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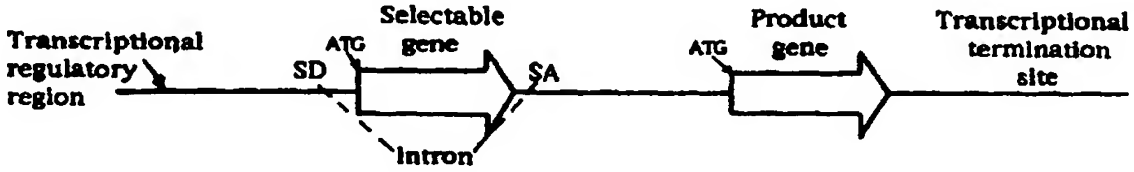

(57) Abstract

A method for selecting recombinant host cells expressing high levels of a desired protein is described. This method utilizes eukaryotic host cells harboring a DNA construct comprising a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene. The selectable gene is positioned within an intron defined by a splice donor site and a splice acceptor site and the selectable gene and product gene are under the transcriptional control of a single transcriptional regulatory region. The splice donor site is generally an efficient splice donor site and thereby regulates expression of the product gene using the transcriptional regulatory region. The transfected cells are cultured so as to express the gene encoding the product in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, whereupon either the desired product is recovered or cells having multiple copies of the product gene are identified.

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METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to a method of selecting for high-expressing host cells, a method of producing a protein of interest in high yields and a method of producing eukaryotic cells having multiple copies of a sequence encoding a protein of interest.

Description of Background and Related Art

The discovery of methods for introducing DNA into living host cells in a functional form has provided the key to understanding many fundamental biological processes, and has made possible the production of important proteins and other molecules in commercially useful quantities.

Despite the general success of such gene transfer methods, several common problems exist that may limit the efficiency with which a gene encoding a desired protein can be introduced into and expressed in a host cell. One problem is knowing when the gene has been successfully transferred into recipient cells. A second problem is distinguishing between those cells that contain the gene and those that have survived the transfer procedures but do not contain the gene. A third problem is identifying and isolating those cells that contain the gene and that are expressing high levels of the protein encoded by the gene.

In general, the known methods for introducing genes into eukaryotic cells tend to be highly inefficient. Of the cells in a given culture, only a small proportion take up and express exogenously added DNA, and an even smaller proportion stably maintain that DNA.

Identification of those cells that have incorporated a product gene encoding a desired protein typically is achieved by introducing into the same cells another gene, commonly referred to as a selectable gene, that encodes a selectable marker. A selectable marker is a protein that is necessary for the growth or survival of a host cell under the particular culture conditions chosen, such as an enzyme that confers resistance to an antibiotic or other drug, or an enzyme that compensates for a metabolic or catabolic defect in the host cell. For example, selectable genes commonly used with eukaryotic cells include the genes for aminoglycoside phosphotransferase (APH), hygromycin phosphotransferase (hyg), dihydrofolate reductase (DHFR), thymidine kinase (tk), neomycin, puromycin, glutamine synthetase, and asparagine synthetase.

The method of identifying a host cell that has incorporated one gene on the basis of expression by the host cell of a second incorporated gene encoding a selectable marker is referred to as cotransfectation (or cotransfection). In that method, a gene encoding a desired polypeptide and a selection gene typically are introduced into the host cell simultaneously, although they may be introduced sequentially. In the case of simultaneous cotransfectation, the gene encoding the desired polypeptide

and the selectable gene may be present on a single DNA molecule or on separate DNA molecules prior to being introduced into the host cells. Wigler *et al.*, Cell, 16:777 (1979). Cells that have incorporated the gene encoding the desired polypeptide then are identified or isolated by culturing the cells under conditions that preferentially allow for the growth or survival of those cells that synthesize the selectable marker encoded by the selectable gene.

The level of expression of a gene introduced into a eukaryotic host cell depends on multiple factors, including gene copy number, efficiency of transcription, messenger RNA (mRNA) processing, stability, and translation efficiency. Accordingly, high level expression of a desired polypeptide typically will involve optimizing one or more of those factors.

For example, the level of protein production may be increased by covalently joining the coding sequence of the gene to a "strong" promoter or enhancer that will give high levels of transcription. Promoters and enhancers are nucleotide sequences that interact specifically with proteins in a host cell that are involved in transcription. Kriegler, Meth. Enzymol., 185:512 (1990); Maniatis *et al.*, Science, 236:1237 (1987). Promoters are located upstream of the coding sequence of a gene and facilitate transcription of the gene by RNA polymerase. Among the eukaryotic promoters that have been identified as strong promoters for high-level expression are the SV40 early promoter, adenovirus major late promoter, mouse metallothionein-I promoter, Rous sarcoma virus long terminal repeat, and human cytomegalovirus immediate early promoter (CMV).

Enhancers stimulate transcription from a linked promoter. Unlike promoters, enhancers are active when placed downstream from the transcription initiation site or at considerable distances from the promoter, although in practice enhancers may overlap physically and functionally with promoters. For example, all of the strong promoters listed above also contain strong enhancers. Bendig, Genetic Engineering, 7:91 (Academic Press, 1988).

The level of protein production also may be increased by increasing the gene copy number in the host cell. One method for obtaining high gene copy number is to directly introduce into the host cell multiple copies of the gene, for example, by using a large molar excess of the product gene relative to the selectable gene during cotransfectation. Kaufman, Meth. Enzymol., 185:537 (1990). With this method, however, only a small proportion of the cotransfected cells will contain the product gene at high copy number. Furthermore, because no generally applicable, convenient method exists for distinguishing such cells from the majority of cells that contain fewer copies of the product gene, laborious and time-consuming screening methods typically are required to identify the desired high-copy number transfectants.

Another method for obtaining high gene copy number involves cloning the gene in a vector that is capable of replicating autonomously in the host cell. Examples of such vectors include mammalian expression vectors

derived from Epstein-Barr virus or bovine papilloma virus, and yeast 2-micron plasmid vectors. Stephens & Hentschel, Biochem. J., 248:1 (1987); Yates *et al.*, Nature, 313:812 (1985); Beggs, Genetic Engineering, 2:175 (Academic Press, 1981).

Yet another method for obtaining high gene copy number involves gene amplification in the host cell. Gene amplification occurs naturally in eukaryotic cells at a relatively low frequency. Schimke, J. Biol. Chem., 263:5989 (1988). However, gene amplification also may be induced, or at least selected for, by exposing host cells to appropriate selective pressure. For example, in many cases it is possible to introduce a product gene together with an amplifiable gene into a host cell and subsequently select for amplification of the marker gene by exposing the cotransfected cells to sequentially increasing concentrations of a selective agent. Typically the product gene will be coamplified with the marker gene under such conditions.

The most widely used amplifiable gene for that purpose is a DHFR gene, which encodes a dihydrofolate reductase enzyme. The selection agent used in conjunction with a DHFR gene is methotrexate (Mtx). A host cell is cotransfected with a product gene encoding a desired protein and a DHFR gene, and transfectants are identified by first culturing the cells in culture medium that contains Mtx. A suitable host cell when a wild-type DHFR gene is used is the Chinese Hamster Ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub & Chasin, Proc. Nat. Acad. Sci. USA, 77:4216 (1980). The transfected cells then are exposed to successively higher amounts of Mtx. This leads to the synthesis of multiple copies of the DHFR gene, and concomitantly, multiple copies of the product gene. Schimke, J. Biol. Chem., 263:5989 (1988); Axel *et al.*, U.S. Patent No. 4,399,216; Axel *et al.*, U.S. Patent No. 4,634,665. Other references directed to co-transfection of a gene together with a genetic marker that allows for selection and subsequent amplification include Kaufman in Genetic Engineering, ed. J. Setlow (Plenum Press, New York), Vol. 9 (1987); Kaufman and Sharp, J. Mol. Biol., 159:601 (1982); Ringold *et al.*, J. Mol. Appl. Genet., 1:165-175 (1981); Kaufman *et al.*, Mol. Cell Biol., 5:1750-1759 (1985); Kaetzel and Nilson, J. Biol. Chem., 263:6244-6251 (1988); Hung *et al.*, Proc. Natl. Acad. Sci. USA, 83:261-264 (1986); Kaufman *et al.*, EMBO J., 6:87-93 (1987); Johnston and Kucey, Science, 242:1551-1554 (1988); Urlaub *et al.*, Cell, 33:405-412 (1983).

To extend the DHFR amplification method to other cell types, a mutant DHFR gene that encodes a protein with reduced sensitivity to methotrexate may be used in conjunction with host cells that contain normal numbers of an endogenous wild-type DHFR gene. Simonsen and Levinson, Proc. Natl. Acad. Sci. USA, 80:2495 (1983); Wigler *et al.*, Proc. Natl. Acad. Sci. USA, 77:3567-3570 (1980); Haber and Schimke, Somatic Cell Genetics, 8:499-508 (1982).

Alternatively, host cells may be co-transfected with the product gene, a DHFR gene, and a dominant selectable gene, such as a neo^r gene. Kim

and Wold, Cell, 42:129 (1985); Capon *et al.*, U.S. Pat. No. 4,965,199. Transfectants are identified by first culturing the cells in culture medium containing neomycin (or the related drug G418), and the transfectants so identified then are selected for amplification of the DHFR gene and the product gene by exposure to successively increasing amounts of Mtx.

As will be appreciated from this discussion, the selection of recombinant host cells that express high levels of a desired protein generally is a multi-step process. In the first step, initial transfectants are selected that have incorporated the product gene and the selectable gene. In subsequent steps, the initial transfectants are subject to further selection for high-level expression of the selectable gene and then random screening for high-level expression of the product gene. To identify cells expressing high levels of the desired protein, typically one must screen large numbers of transfectants. The majority of transfectants produce less than maximal levels of the desired protein. Further, Mtx resistance in DHFR transformants is at least partially conferred by varying degrees of gene amplification. Schimke, Cell, 37:705-713 (1984). The inadequacies of co-expression of the non-selected gene have been reported by Wold *et al.*, Proc. Natl. Acad. Sci. USA, 76:5684-5688 (1979). Instability of the amplified DNA is reported by Kaufman and Schimke, Mol. Cell Biol., 1:1069-1076 (1981); Haber and Schimke, Cell, 26:355-362 (1981); and Fedespiel *et al.*, J. Biol. Chem., 259:9127-9140 (1984).

Several methods have been described for directly selecting such recombinant host cells in a single step. One strategy involves co-transfecting host cells with a product gene and a DHFR gene, and selecting those cells that express high levels of DHFR by directly culturing in medium containing a high concentration of Mtx. Many of the cells selected in that manner also express the co-transfected product gene at high levels. Page and Sydenham, Bio/Technology, 9:64 (1991). This method for single-step selection suffers from certain drawbacks that limit its usefulness. High-expressing cells obtained by direct culturing in medium containing a high level of a selection agent may have poor growth and stability characteristics, thus limiting their usefulness for long-term production processes. Page and Snyderman, Bio/Technology, 9:64 (1991). Single-step selection for high-level resistance to Mtx may produce cells with an altered, Mtx-resistant DHFR enzyme, or cells that have altered Mtx transport properties, rather than cells containing amplified genes. Haber *et al.*, J. Biol. Chem., 256:9501 (1981); Assaraf and Schimke, Proc. Natl. Acad. Sci. USA, 84:7154 (1987).

Another method involves the use of polycistronic mRNA expression vectors containing a product gene at the 5' end of the transcribed region and a selectable gene at the 3' end. Because translation of the selectable gene at the 3' end of the polycistronic mRNA is inefficient, such vectors exhibit preferential translation of the product gene and require high levels of polycistronic mRNA to survive selection. Kaufman, Meth.

Enzymol., 185:487 (1990); Kaufman, Meth. Enzymol., 185:537 (1990); Kaufman *et al.*, EMBO J., 6:187 (1987). Accordingly, cells expressing high levels of the desired protein product may be obtained in a single step by culturing the initial transfectants in medium containing a selection agent appropriate for use with the particular selectable gene. However, the utility of these vectors is variable because of the unpredictable influence of the upstream product reading frame on selectable marker translation and because the upstream reading frame sometimes becomes deleted during methotrexate amplification (Kaufman *et al.*, J. Mol. Biol., 159:601-621 [1982]; Levinson, Methods in Enzymology, San Diego: Academic Press, Inc. [1990]). Later vectors incorporated an internal translation initiation site derived from members of the picornavirus family which is positioned between the product gene and the selectable gene (Pelletier *et al.*, Nature, 334:320 [1988]; Jang *et al.*, J. Virol., 63:1651 [1989]).

A third method for single-step selection involves use of a DNA construct with a selectable gene containing an intron within which is located a gene encoding the protein of interest. See U.S. Patent No. 5,043,270 and Abrams *et al.*, J. Biol. Chem., 264(24): 14016-14021 (1989). In yet another single-step selection method, host cells are co-transfected with an intron-modified selectable gene and a gene encoding the protein of interest. See WO 92/17566, published October 15, 1992. The intron-modified gene is prepared by inserting into the transcribed region of a selectable gene an intron of such length that the intron is correctly spliced from the corresponding mRNA precursor at low efficiency, so that the amount of selectable marker produced from the intron-modified selectable gene is substantially less than that produced from the starting selectable gene. These vectors help to insure the integrity of the integrated DNA construct, but transcriptional linkage is not achieved as selectable gene and the protein gene are driven by separate promoters.

Other mammalian expression vectors that have single transcription units have been described. Retroviral vectors have been constructed (Cepko *et al.*, Cell, 37:1053-1062 [1984]) in which a cDNA is inserted between the endogenous Moloney murine leukemia virus (M-MuLV) splice donor and splice acceptor sites which are followed by a neomycin resistance gene. This vector has been used to express a variety of gene products following retroviral infection of several cell types.

With the above drawbacks in mind, it is one object of the present invention to increase the level of homogeneity with regard to expression levels of stable clones transfected with a product gene of interest, by expressing a selectable marker (DHFR) and the protein of interest from a single promoter.

It is another object to provide a method for selecting stable, recombinant host cells that express high levels of a desired protein product, which method is rapid and convenient to perform, and reduces the numbers of transfected cells which need to be screened. Furthermore, it is

an object to allow high levels of single and two unit polypeptides to be rapidly generated from clones or pools of stable host cell transfectants.

It is an additional object to provide expression vectors which bias for active integration events (*i.e.* have an increased tendency to generate transformants wherein the DNA construct is inserted into a region of the genome of the host cell which results in high level expression of the product gene) and can accommodate a variety of product genes without the need for modification.

SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to a DNA construct (DNA molecule) alternative terminology comprising a 5' transcriptional initiation site and a 3' transcriptional termination site, a selectable gene (preferably an amplifiable gene) and a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene positioned within an intron defined by a splice donor site and a splice acceptor site. The splice donor site preferably comprises an effective splice donor sequence as herein defined and thereby regulates expression of the product gene using the transcriptional regulatory region.

In another embodiment, the invention provides a method for producing a product of interest comprising culturing a eukaryotic cell which has been transfected with the DNA construct described above, so as to express the product gene and recovering the product.

In a further embodiment, the invention provides a method for producing eukaryotic cells having multiple copies of the product gene comprising transfecting eukaryotic cells with the DNA construct described above (where the selectable gene is an amplifiable gene), growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene. Preferably transfection of the cells is achieved using electroporation.

After transfection of the host cells, most of the transfectants fail to exhibit the selectable phenotype characteristic of the protein encoded by the selectable gene, but surprisingly a small proportion of the transfectants do exhibit the selectable phenotype, and among those transfectants, the majority are found to express high levels of the desired product encoded by the product gene. Thus, the invention provides an improved method for the selection of recombinant host cells expressing high levels of a desired product, which method is useful with a wide variety of eukaryotic host cells and avoids the problems inherent in existing cell selection technology.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1D illustrate schematically various DNA constructs encompassed by the instant invention. The large arrows represent the selectable gene and the product gene, the V formed by the dashed lines shows the region of the precursor RNA internal to the 5' splice donor site (SD) and 3' splice acceptor site (SA) that is excised from vectors that contain a functional SD. The transcriptional regulatory region, selectable gene, product gene and transcriptional termination site are depicted in Figure 1A. Figure 1B depicts the DNA constructs of Example 1. The various splice donor sequences are depicted, *i.e.*, wild type ras splice donor sequence (WT ras), mutant ras splice donor sequence (MUTANT ras) and non-functional splice donor sequence (▲GT). The probes used for Northern blot analysis in Example 1 are shown in Figure 1B. Figure 1C depicts the DNA constructs of Example 2 and Figure 1D depicts the DNA construct of Example 3 used for expression of anti-IgE V_H.

Figure 2 depicts schematically the control DNA construct used in Example 1.

Figures 3A-Q depict the nucleotide sequence (SEQ ID NO: 1) of the DHFR/intron-(WT ras SD)-tPA expression vector of Example 1.

Figure 4 is a bar graph which shows the number of colonies that form in selective medium after electroporation of linearized duplicate miniprep DNA's prepared in parallel from the three vectors shown in Figure 1B (*i.e.* with wild type ras splice donor sequence [WT ras], mutant ras splice donor sequence [MUTANT ras] and non-functional splice donor sequence [▲GT]) and from the control vector that has DHFR under control of SV40 promoter and tPA under control of CMV promoter (see Figure 2). Cells were selected in nucleoside free medium and counted with an automated colony counter.

Figures 5A-C are bar graphs depicting expression of tPA from stable pools and clones generated from the vectors shown in Figure 1B. In Figure 5A greater than 100 clones from each vector transfection were mixed, plated in 24 well plates, and assayed by tPA ELISA at "saturation". In Figure 5B, twenty clones chosen at random derived from each of the vectors were assayed by tPA ELISA at "saturation". In Figure 5C, the pools mentioned in Figure 5A (except the ▲GT pool) were exposed to 200nM Mtx to select for DHFR amplification and then pooled and assayed for tPA expression.

Figures 6A-P depict the nucleotide sequence (SEQ ID NO: 2) of the DHFR/intron-(WT ras SD)-TNFr-IgG expression vector of Example 2.

Figures 7A-B are bar graphs depicting expression of TNFr-IgG using dicistronic or control vectors (see Example 2). Vectors containing TNFr-IgG (but otherwise identical to those described for tPA expression in Example 1) were constructed (see Figure 1C), introduced into dp12.CHO cells by electroporation, pooled, and assayed for product expression before (Figure 7A) and after (Figure 7B) being subjected to amplification in 200nM Mtx.

Figure 8 depicts schematically the DNA construct used for expression of the V_L of anti-IgE in Example 3.

Figures 9A-O depict the nucleotide sequence (SEQ ID NO: 3) of the anti-IgE V_H expression vector of Example 3.

Figures 10A-Q depict the nucleotide sequence (SEQ ID NO: 4) of the anti-IgE V_L expression vector of Example 3.

Figure 11 is a bar graph depicting anti-IgE expression in Example 3. Heavy (V_H) and light (V_L) chain expression vectors were constructed, co-electroporated into CHO cells, clones were selected and assayed for antibody expression. Additionally, pools were established and assessed with regard to expression before and after Mtx selection at 200nM and 1μM.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions:

The "DNA construct" disclosed herein comprises a non-naturally occurring DNA molecule which can either be provided as an isolate or integrated in another DNA molecule *e.g.* in an expression vector or the chromosome of an eukaryotic host cell.

The term "selectable gene" as used herein refers to a DNA that encodes a selectable marker necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. Accordingly, a host cell that is transformed with a selectable gene will be capable of growth or survival under certain cell culture conditions wherein a non-transfected host cell is not capable of growth or survival. Typically, a selectable gene will confer resistance to a drug or compensate for a metabolic or catabolic defect in the host cell. Examples of selectable genes are provided in the following table. See also Kaufman, Methods in Enzymology, 185: 537-566 (1990), for a review of these.

TABLE 1

Selectable Genes and their Selection Agents

Selection Agent	Selectable Gene
Methotrexate	Dihydrofolate reductase
Cadmium	Metallothionein
PALA	CAD
Xyl-A-or adenosine and 2'-deoxycoformycin	Adenosine deaminase
Adenine, azaserine, and coformycin	Adenylate deaminase
6-Azauridine, pyrazofuran	UMP Synthetase
Mycophenolic acid	IMP 5'-dehydrogenase

Mycophenolic acid with limiting xanthine	Xanthine-guanine phosphoribosyltransferase
Hypoxanthine, aminopterin, and thymidine (HAT)	Mutant HGPRTase or mutant thymidine kinase
5-Fluorodeoxyuridine	Thymidylate synthetase
Multiple drugs *e.g.* adriamycin, vincristine or colchicine	P-glycoprotein 170
Aphidicolin	Ribonucleotide reductase
Methionine sulfoximine	Glutamine synthetase
β-Aspartyl hydroxamate or Albizziin	Asparagine synthetase
Canavanine	Arginosuccinate synthetase
α-Difluoromethylornithine	Ornithine decarboxylase
Compactin	HMG-CoA reductase
Tunicamycin	*N*-Acetylglucosaminyl transferase
Borrelidin	Threonyl-tRNA synthetase
Ouabain	Na^+K^+-ATPase

The preferred selectable gene is an amplifiable gene. As used herein, the term "amplifiable gene" refers to a gene which is amplified (*i.e.* additional copies of the gene are generated which survive in intrachromosomal or extrachromosomal form) under certain conditions. The amplifiable gene usually encodes an enzyme (*i.e.* an amplifiable marker) which is required for growth of eukaryotic cells under those conditions. For example, the gene may encode DHFR which is amplified when a host cell transformed therewith is grown in Mtx. According to Kaufman, the selectable genes in Table 1 above can also be considered amplifiable genes. An example of a selectable gene which is generally not considered to be an amplifiable gene is the neomycin resistance gene (Cepko *et al.*, *supra*).

As used herein, "selective medium" refers to nutrient solution used for growing eukaryotic cells which have the selectable gene and therefore includes a "selection agent". Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ([MEM], Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ([DMEM], Sigma) are exemplary nutrient solutions. In addition, any of the media described in Ham and Wallace, Meth. Enz., 58:44 (1979), Barnes and Sato, Anal. Biochem., 102:255

(1980), U.S. Patent Nos. 4,767,704; 4,657,866; 4,927,762; or 4,560,655; WO 90/03430; WO 87/00195; U.S. Patent Re. 30,985; or U.S. Patent No. 5,122,469, the disclosures of all of which are incorporated herein by reference, may be used as culture media. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The preferred nutrient solution comprises fetal bovine serum.

The term "selection agent" refers to a substance that interferes with the growth or survival of a host cell that is deficient in a particular selectable gene. Examples of selection agents are presented in Table 1 above. The selection agent preferably comprises an "amplifying agent" which is defined for purposes herein as an agent for amplifying copies of the amplifiable gene, such as Mtx if the amplifiable gene is DHFR. See Table 1 for examples of amplifying agents.

As used herein, the term "transcriptional initiation site" refers to the nucleic acid in the DNA construct corresponding to the first nucleic acid incorporated into the primary transcript, *i.e.*, the mRNA precursor, which site is generally provided at, or adjacent to, the 5' end of the DNA construct.

The term "transcriptional termination site" refers to a sequence of DNA, normally represented at the 3' end of the DNA construct, that causes RNA polymerase to terminate transcription.

As used herein, "transcriptional regulatory region" refers to a region of the DNA construct that regulates transcription of the selectable gene and the product gene. The transcriptional regulatory region normally refers to a promoter sequence (*i.e.* a region of DNA involved in binding of RNA polymerase to initiate transcription) which can be constitutive or inducible and, optionally, an enhancer (*i.e.* a *cis*-acting DNA element, usually from about 10-300 bp, that acts on a promoter to increase its transcription).

As used herein, "product gene" refers to DNA that encodes a desired protein or polypeptide product. Any product gene that is capable of expression in a host cell may be used, although the methods of the invention are particularly suited for obtaining high-level expression of a product gene that is not also a selectable or amplifiable gene. Accordingly, the protein or polypeptide encoded by a product gene typically will be one that is not necessary for the growth or survival of a host cell under the particular cell culture conditions chosen. For example, product genes suitably encode a peptide, or may encode a polypeptide sequence of

amino acids for which the chain length is sufficient to produce higher levels of tertiary and/or quaternary structure.

Examples of bacterial polypeptides or proteins include, *e.g.*, alkaline phosphatase and β-lactamase. Examples of mammalian polypeptides or proteins include molecules such as renin; a growth hormone, including human growth hormone, and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.*, M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.*, IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; antibodies; chimeric proteins such as immunoadhesins and fragments of any of the above-listed polypeptides.

The product gene preferably does not consist of an anti-sense sequence for inhibiting the expression of a gene present in the host. Preferred proteins herein are therapeutic proteins such as TGF-β, TGF-α, PDGF, EGF, FGF, IGF-I, DNase, plasminogen activators such as t-PA, clotting factors such as tissue factor and factor VIII, hormones such as relaxin and insulin, cytokines such as IFN-γ, chimeric proteins such as TNF receptor IgG immunoadhesin (TNFr-IgG) or antibodies such as anti-IgE.

The term "intron" as used herein refers to a nucleotide sequence present within the transcribed region of a gene or within a messenger RNA precursor, which nucleotide sequence is capable of being excised, or spliced, from the messenger RNA precursor by a host cell prior to translation. Introns suitable for use in the present invention are suitably prepared by any of several methods that are well known in the art, such as purification from a naturally occurring nucleic acid or *de novo* synthesis. The introns present in many naturally occurring eukaryotic genes have been identified and characterized. Mount, Nuc. Acids Res., 10:459 (1982). Artificial introns comprising functional splice sites also have been described. Winey *et al.*, Mol. Cell Biol., 9:329 (1989); Gatermann *et al.*, Mol. Cell Biol., 9:1526 (1989). Introns may be obtained from naturally occurring nucleic acids, for example, by digestion of a naturally occurring nucleic acid with a suitable restriction endonuclease, or by PCR cloning using primers complementary to sequences at the 5' and 3' ends of the intron. Alternatively, introns of defined sequence and length may be prepared synthetically using various methods in organic chemistry. Narang *et al.*, Meth. Enzymol., 68:90 (1979); Caruthers *et al.*, Meth. Enzymol., 154:287 (1985); Froehler *et al.*, Nuc. Acids Res., 14:5399 (1986).

As used herein "splice donor site" or "SD" refers to the DNA sequence immediately surrounding the exon-intron boundary at the 5' end of the intron, where the "exon" comprises the nucleic acid 5' to the intron. Many splice donor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. An "efficient splice donor sequence" refers to a nucleic acid sequence encoding a splice donor site wherein the efficiency of splicing of messenger RNA precursors having the splice donor sequence is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. Examples of efficient splice donor sequences include the wild type (WT) ras splice donor sequence and the GAC:GTAAGT sequence of Example 3. Other efficient splice donor sequences can be readily selected using the techniques for measuring the efficiency of splicing disclosed herein.

The terms "PCR" and "polymerase chain reaction" as used herein refer to the *in vitro* amplification method described in US Patent No. 4,683,195 (issued July 28, 1987). In general, the PCR method involves repeated cycles of primer extension synthesis, using two DNA primers capable of hybridizing preferentially to a template nucleic acid comprising the nucleotide sequence to be amplified. The PCR method can be used to clone specific DNA sequences from total genomic DNA, cDNA transcribed from cellular RNA, viral or plasmid DNAs. Wang & Mark, in PCR Protocols, pp.70-75 (Academic Press, 1990); Scharf, in PCR Protocols, pp. 84-98; Kawasaki & Wang, in PCR Technology, pp. 89-97 (Stockton Press, 1989). Reverse transcription-polymerasechain reaction (RT-PCR) can be used to analyze RNA samples containing mixtures of spliced and unspliced mRNA transcripts. Fluorescently tagged primers designed to span the intron are used to

amplify both spliced and unspliced targets. The resultant amplification products are then separated by gel electrophoresis and quantitated by measuring the fluorescent emission of the appropriate band(s). A comparison is made to determine the amount of spliced and unspliced transcripts present in the RNA sample.

One preferred splice donor sequence is a "consensus splice donor sequence". The nucleotide sequences surrounding intron splice sites, which sequences are evolutionarily highly conserved, are referred to as "consensus splice donor sequences". In the mRNAs of higher eukaryotes, the 5' splice site occurs within the consensus sequence AG:GUAAGU (wherein the colon denotes the site of cleavage and ligation). In the mRNAs of yeast, the 5' splice site is bounded by the consensus sequence :GUAUGU. Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986).

The expression "splice acceptor site" or "SA" refers to the sequence immediately surrounding the intron-exon boundary at the 3' end of the intron, where the "exon" comprises the nucleic acid 3' to the intron. Many splice acceptor sites have been characterized and Ohshima *et al.*, J. Mol. Biol., 195:247-259 (1987) provides a review of these. The preferred splice acceptor site is an efficient splice acceptor site which refers to a nucleic acid sequence encoding a splice acceptor site wherein the efficiency of splicing of messenger RNA precursors having the splice acceptor site is between about 80 to 99% and preferably 90 to 95% as determined by quantitative PCR. The splice acceptor site may comprise a consensus sequence. In the mRNAs of higher eukaryotes, the 3' splice acceptor site occurs within the consensus sequence $(U/C)_{11}$NCAG:G. In the mRNAs of yeast, the 3' acceptor splice site is bounded by the consensus sequence (C/U)AG:. Padgett, *et al.*, *supra*.

As used herein "culturing for sufficient time to allow amplification to occur" refers to the act of physically culturing the eukaryotic host cells which have been transformed with the DNA construct in cell culture media containing the amplifying agent, until the copy number of the amplifiable gene (and preferably also the copy number of the product gene) in the host cells has increased relative to the transformed cells prior to this culturing.

The term "expression" as used herein refers to transcription or translation occurring within a host cell. The level of expression of a product gene in a host cell may be determined on the basis of either the amount of corresponding mRNA that is present in the cell or the amount of the protein encoded by the product gene that is produced by the cell. For example, mRNA transcribed from a product gene is desirably quantitated by northern hybridization. Sambrook, *et al.*, Molecular Cloning: A Laboratory Manual, pp. 7.3-7.57 (Cold Spring Harbor Laboratory Press, 1989). Protein encoded by a product gene can be quantitated either by assaying for the biological activity of the protein or by employing assays that are independent of such activity, such as western blotting or radioimmunoassay using antibodies that are capable of reacting with the protein. Sambrook,

et al., Molecular Cloning: A Laboratory Manual, pp. 18.1-18.88 (Cold Spring Harbor Laboratory Press, 1989).

Modes for Carrying Out the Invention

Methods and compositions are provided for enhancing the stability and/or copy number of a transcribed sequence in order to allow for elevated levels of a RNA sequence of interest. In general, the methods of the present invention involve transfecting a eukaryotic host cell with an expression vector comprising both a product gene encoding a desired polypeptide and a selectable gene (preferably an amplifiable gene).

Selectable genes and product genes may be obtained from genomic DNA, cDNA transcribed from cellular RNA, or by *in vitro* synthesis. For example, libraries are screened with probes (such as antibodies or oligonucleotides of about 20-80 bases) designed to identify the selectable gene or the product gene (or the protein(s) encoded thereby). Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the selectable gene or product gene is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various tissues known to contain the selectable gene or product gene. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide generally is labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use ^{32}P- labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Sometimes, the DNA encoding the selectable gene and product gene is preceded by DNA encoding a signal sequence having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the expression vector, or it may be a part of the selectable gene or product gene that is inserted into the expression vector. If a heterologous signal sequence is used, it preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* α-factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence

of the protein of interest is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. The DNA for such precursor region is ligated in reading frame to the selectable gene or product gene.

As shown in Figure 1A, the selectable gene is generally provided at the 5' end of the DNA construct and this selectable gene is followed by the product gene. Therefore, the full length (non-spiced) message will contain DHFR as the first open reading frame and will therefore generate DHFR protein to allow selection of stable transfectants. The full length message is not expected to generate appreciable amounts of the protein of interest as the second AUG in a dicistronic message is an inefficient initiator of translation in mammalian cells (Kozak, J. Cell Biol., 115: 887-903 [1991]).

The selectable gene is positioned within an intron. Introns are noncoding nucleotide sequences, normally present within many eukaryotic genes, which are removed from newly transcribed mRNA precursors in a multiple-step process collectively referred to as splicing.

A single mechanism is thought to be responsible for the splicing of mRNA precursors in mammalian, plant, and yeast cells. In general, the process of splicing requires that the 5' and 3' ends of the intron be correctly cleaved and the resulting ends of the mRNA be accurately joined, such that a mature mRNA having the proper reading frame for protein synthesis is produced. Analysis of a variety of naturally occurring and synthetically constructed mutant genes has shown that nucleotide changes at many of the positions within the consensus sequences at the 5' and 3' splice sites have the effect of reducing or abolishing the synthesis of mature mRNA. Sharp, Science, 235:766 (1987); Padgett, *et al.*, Ann. Rev. Biochem., 55:1119 (1986); Green, Ann. Rev. Genet., 20:671 (1986). Mutational studies also have shown that RNA secondary structures involving splicing sites can affect the efficiency of splicing. Solnick, Cell, 43:667 (1985); Konarska, *et al.*, Cell, 42:165 (1985).

The length of the intron may also affect the efficiency of splicing. By making deletion mutations of different sizes within the large intron of the rabbit beta-globin gene, Wieringa, *et al.* determined that the minimum intron length necessary for correct splicing is about 69 nucleotides. Cell, 37:915 (1984). Similar studies of the intron of the adenovirus E1A region have shown that an intron length of about 78 nucleotides allows correct splicing to occur, but at reduced efficiency. Increasing the length of the intron to 91 nucleotides restores normal splicing efficiency, whereas truncating the intron to 63 nucleotides abolishes correct splicing. Ulfendahl, *et al.*, Nuc. Acids Res., 13:6299 (1985).

To be useful in the invention, the intron must have a length such that splicing of the intron from the mRNA is efficient. The preparation of introns of differing lengths is a routine matter, involving methods well known in the art, such as *de novo* synthesis or *in vitro* deletion

mutagenesis of an existing intron. Typically, the intron will have a length of at least about 150 nucleotides, since introns which are shorter than this tend to be spliced less efficiently. The upper limit for the length of the intron can be up to 30 kB or more. However, as a general proposition, the intron is generally less than about 10 kB in length.

The intron is modified to contain the selectable gene not normally present within the intron using any of the various known methods for modifying a nucleic acid *in vitro*. Typically, a selectable gene will be introduced into an intron by first cleaving the intron with a restriction endonuclease, and then covalently joining the resulting restriction fragments to the selectable gene in the correct orientation for host cell expression, for example by ligation with a DNA ligase enzyme.

The DNA construct is dicistronic, *i.e.* the selectable gene and product gene are both under the transcriptional control of a single transcriptional regulatory region. As mentioned above, the transcriptional regulatory region comprises a promoter. Suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, J. Biol. Chem., 255:2073 [1980]) or other glycolytic enzymes (Hess *et al.*, J. Adv. Enzyme Reg., 7:149 [1968]; and Holland, Biochemistry, 17:4900 [1978]), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in Hitzeman *et al.*, EP 73,657A. Yeast enhancers also are advantageously used with yeast promoters.

Expression control sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide.

Product gene transcription from vectors in mammalian host cells is controlled by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g. the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the product gene, provided such promoters are compatible with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. Fiers *et al.*, Nature, 273:113 (1978); Mulligan and Berg, Science, 209:1422-1427 (1980); Pavlakis *et al.*, Proc. Natl. Acad. Sci. USA, 78:7398-7402 (1981). The immediate early promoter of the human cytomegalovirus (CMV) is conveniently obtained as a *Hin*dIII E restriction fragment. Greenaway *et al.*, Gene, 18:355-360 (1982). A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. 4,419,446. A modification of this system is described in U.S. 4,601,978. See also Gray *et al.*, Nature, 295:503-508 (1982) on expressing cDNA encoding immune interferon in monkey cells; , Reyes *et al.*, Nature, 297:598-601 (1982) on expression of human β-interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus, Canaani and Berg, Proc. Natl. Acad. Sci. USA, 79:5166-5170 (1982) on expression of the human interferon β1 gene in cultured mouse and rabbit cells, and Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 79:6777-6781 (1982) on expression of bacterial CAT sequences in CV-1 monkey kidney cells, chicken embryo fibroblasts, Chinese hamster ovary cells, HeLa cells, and mouse NIH-3T3 cells using the Rous sarcoma virus long terminal repeat as a promoter.

Preferably the transcriptional regulatory region in higher eukaryotes comprises an enhancer sequence. Enhancers are relatively orientation and position independent having been found 5' (Lainins *et al.*, Proc. Natl. Acad. Sci. USA, 78:993 [1981]) and 3' (Lusky *et al.*, Mol. Cell Bio., 3:1108 [1983]) to the transcription unit, within an intron (Banerji *et al.*, Cell, 33:729 [1983]) as well as within the coding sequence itself (Osborne *et al.*, Mol. Cell Bio., 4:1293 [1984]). Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer (CMV), the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, Nature, 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the product gene, but is preferably located at a site 5' from the promoter.

The DNA construct has a transcriptional initiation site following the transcriptional regulatory region and a transcriptional termination region following the product gene (see Figure 1A). These sequences are provided in the DNA construct using techniques which are well known in the art.

The DNA construct normally forms part of an expression vector which may have other components such as an origin of replication (*i.e.*, a nucleic acid sequence that enables the vector to replicate in one or more selected host cells) and, if desired, one or more additional selectable gene(s). Construction of suitable vectors containing the desired coding and control sequences employs standard ligation techniques. Isolated plasmids or DNA

fragments are cleaved, tailored, and religated in the form desired to generate the plasmids required.

Generally, in cloning vectors the origin of replication is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known. The 2μ plasmid origin of replication is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

For analysis to confirm correct sequences in plasmids constructed, plasmids from the transformants are prepared, analyzed by restriction, and/or sequenced by the method of Messing *et al.*, Nucleic Acids Res., 9:309 (1981) or by the method of Maxam *et al.*, Methods in Enzymology, 65:499 (1980).

The expression vector having the DNA construct prepared as discussed above is transformed into a eukaryotic host cell. Suitable host cells for cloning or expressing the vectors herein are yeast or higher eukaryote cells.

Eukaryotic microbes such as filamentous fungi or yeast are suitable hosts for vectors containing the product gene. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *S. pombe* [Beach and Nurse, Nature, 290:140 (1981)], *Kluyveromyces lactis* [Louvencourt *et al.*, J. Bacteriol., 737 (1983)], *yarrowia* [EP 402,226], *Pichia pastoris* [EP 183,070], *Trichoderma reesia* [EP 244,234], *Neurospora crassa* [Case *et al.*, Proc. Natl. Acad. Sci. USA, 76:5259-5263 (1979)], and *Aspergillus* hosts such as *A. nidulans* [Ballance *et al.*, Biochem. Biophys. Res. Commun., 112:284-289 (1983); Tilburn *et al.*, Gene, 26:205-221 (1983); Yelton *et al.*, Proc. Natl. Acad. Sci. USA, 81:1470-1474 (1984)] and *A. niger* [Kelly and Hynes, EMBO J., 4:475-479 (1985)].

Suitable host cells for the expression of the product gene are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda*

(caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosphila melanogaster* (fruitfly), and *Bombyx mori* host cells have been identified. See, e.g., Luckow *et al.*, Bio/Technology, 6:47-55 (1988); Miller *et al.*, in Genetic Engineering, Setlow, J.K. *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, Nature, 315:592-594 (1985). A variety of such viral strains are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the product gene. During incubation of the plant cell culture with *A. tumefaciens*, the product gene is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the product gene. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, J. Mol. Appl. Gen., 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA *780* gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years [Tissue Culture, Academic Press, Kruse and Patterson, editors (1973)]. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, J. Gen Virol., 36:59 [1977]); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, Proc. Natl. Acad. Sci. USA, 77:4216 [1980]); dp12.CHO cells (EP 307,247 published 15 March 1989); mouse sertoli cells (TM4, Mather, Biol. Reprod., 23:243-251 [1980]); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, Annals N.Y. Acad. Sci., 383:44-68 [1982]); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transformed with the above-described expression or cloning vectors of this invention and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be used. General aspects of mammalian cell host system transformations have been described by Axel in U.S. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, J. Bact., 130:946 (1977) and Hsiao *et al.*, Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

In the preferred embodiment the DNA is introduced into the host cells using electroporation. See Andreason, J. Tiss. Cult. Meth., 15:56-62 (1993), for a review of electroporation techniques useful for practicing the instantly claimed invention. It was discovered that electroporation techniques for introducing the DNA construct into the host cells were preferable over calcium phosphate precipitation techniques insofar as the latter could cause the DNA to break up and forming concantemers.

The mammalian host cells used to express the product gene herein may be cultured in a variety of media as discussed in the definitions section above. The media contains the selection agent used for selecting transformed host cells which have taken up the DNA construct (either as an intra- or extra-chromosomal element). To achieve selection of the transformed eukaryotic cells, the host cells may be grown in cell culture plates and individual colonies expressing the selectable gene (and thus the product gene) can be isolated and grown in growth medium until the nutrients are depleted. The host cells are then analyzed for transcription and/or transformation as discussed below. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, Proc. Natl. Acad. Sci. USA, 77:5201-5205 [1980]), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly ^{32}P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescens, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the

formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al.*, Am. J. Clin. Path., 75:734-738 (1980).

In the preferred embodiment, the mRNA is analyzed by quantitative PCR (to determine the efficiency of splicing) and protein expression is measured using ELISA as described in Example 1 herein.

The product of interest preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates when directly expressed without a secretory signal. When the product gene is expressed in a recombinant cell other than one of human origin, the product of interest is completely free of proteins or polypeptides of human origin. However, it is necessary to purify the product of interest from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to the product of interest. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. The product of interest thereafter is purified from contaminant soluble proteins and polypeptides, for example, by fractionation on immunoaffinity or ion-exchange columns; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel electrophoresis using, for example, Sephadex G-75; chromatography on plasminogen columns to bind the product of interest and protein A Sepharose columns to remove contaminants such as IgG.

The following examples are offered by way of illustration only and are not intended to limit the invention in any manner. All patent and literature references cited herein are expressly incorporated by reference.

EXAMPLE 1

tPA production using the dicistronic expression vectors

It was sought to increase the level of homogeneity with regard to expression levels of stable clones by expressing a selectable marker (such as DHFR) and the protein of interest from a single promoter. These vectors divert most of the transcript to product expression while linking it at a fixed ratio to DHFR expression via differential splicing.

Vectors were constructed which were derived from the vector pRK (Suva *et al.*, Science, 237:893-896 [1987]) which contains an intron between the cytomegalovirus immediate early promoter (CMV) and the cDNA that encodes

the polypeptide of interest. The intron of pRK is 139 nucleotides in length, has a splice donor site derived from cytomegalovirus immediate early gene (CMVIE), and a splice acceptor site from an IgG heavy chain variable region (V_H) gene (Eaton et al., Biochem., 25:8343 [1986]).

DHFR/intron vectors were constructed by inserting an EcoRV linker into the BSTX1 site present in the intron of pRK7. An 830 base-pair fragment containing a mouse DHFR coding fragment was inserted to obtain DHFR intron expression vectors which differ only in the sequence that comprises the splice donor site. Those sequences were altered by overlapping PCR mutagenesis to obtain sequences that match splice donor sites found between exons 3 and 4 of normal and mutant Ras genes. PCR was also used to destroy the splice donor site.

A mouse DHFR cDNA fragment (Simonsen et al., Proc. Natl. Acad. Sci. USA, 80:2495-2499 [1983]) was inserted into the intron of this vector 59 nucleotides downstream of the splice donor site. The splice donor site of this vector was altered by mutagenesis to change the ratio of spliced to non-spliced message in transfected cells. It has previously been shown that a single nucleotide change (G to A) converted a relatively efficient splice donor site found in the normal ras gene into an inefficient splice site (Cohen et al., Nature, 334:119-124 [1988]). This effect has been demonstrated in the context of the ras gene and confirmed when these sequences were transferred to human growth hormone constructs (Cohen et al., Cell, 58:461-472 [1989]). Additionally, a non functional 5' splice site (GT to CA) was constructed as a control (ΔGT). A polylinker was inserted 35 nucleotides downstream of the 3' splice site to accept the cDNA of interest. A vector containing tPA (Pennica et al., Nature, 301:214-221 [1983]) was linearized downstream of the polyadenylation site before it was introduced into CHO cells (Potter et al., Proc. Natl. Acad. Sci. USA, 81:7161 [1984]).

Plasmid DNA's that contained DHFR/intron, tPA and (a) wild type ras (WT ras), i.e. Figure 3 (SEQ ID NO: 1), (b) mutant ras, or (c) non-functional splice donor site (ΔGT) were introduced into CHO DHFR minus cells by electroporation. The intron vectors were each linearized downstream of the polyadenylation site by restriction endonuclease treatment. The control vector was linearized downstream of the second polyadenylation site. The DNA's were ethanol precipitated after phenol/chloroform extraction and were resuspended in 20μl 1/10 Tris EDTA. Then, 10μg of DNA was incubated with 10^7 CHO.dp12 cells (EP 307,247 published 15 March 1989) in 1 ml of PBS on ice for 10 min. before electroporation at 400 volts and 330μf using a BRL Cell Porator.

Cells were returned to ice for 10 min. before being plated into non-selective medium. After 24 hours cells were fed nucleoside-free medium to select for stable DHFR+ clones which were pooled. The pooled DHFR+ clones were lysed and mRNA's were prepared.

To prepare the mRNA, RNA was extracted from 5 x 10^7 cells which were grown from pools of more than 200 clones derived from the stable

transfection of the three vectors, the essential construction of which is shown in Figure 1B and from non-transfected CHO cells. RNA was purified over oligo-DT cellulase (Collaborative Biomedical Products). 10μg of mRNA was then subjected to Northern blotting which involved running the mRNA on a 1.2% agarose, 6.6% formaldehyde gel, and transferring it to a nylon filter (Stratagene Duralon-UV membrane), prehybridized, probed and washed according to the manufacturer's instructions.

The filter was probed sequentially using probes (shown in Figure 1B) that would detect (a) the full length message, (b) both full length and spliced message, or (c) beta actin. Probing with the long probe showed that the vector that contains the efficient splice donor site (*i.e.* WT ras) generates predominately a mRNA of the size predicted for the spliced product while the other two vectors gave rise primarily to a mRNA that corresponds in size to non-spliced message. The DHFR probe detected only full length message and demonstrated that the WT ras splice donor derived vector generates very little full length message with which to confer a DHFR positive phenotype.

Figure 4 shows the number of DHFR positive colonies obtained after duplicate electroporations with the three intron vectors described above and from a conventional vector that has a CMV promoter driving tPA and a SV40 promoter driving DHFR (see Figure 2). The increase in colony number parallels the increase in full length message that accumulates with the modification of the splice donor sites. The conventional vector efficiently generates colonies and does not vary significantly from the ΔGT construct.

The level of tPA expression was determined by seeding cells in 1 ml of F12:DMEM (50:50, with 5% FBS) in 24 well dishes to near confluency. Growth of the cells continued until the media was exhausted. Media was then assayed by ELISA for tPA production. Briefly, anti-tPA antibody was coated onto the wells of an ELISA microtiter plate, media samples were added to the wells followed by washing. Binding of the antigen (tPA) was then quantified using horse radish peroxidase (HRPO) labelled anti-tPA antibody.

Figure 5A depicts the titers of secreted tPA protein after pooling the clones of each group shown in Figure 4. While the number of colonies increased with a weakening of splice donor function, the inverse was seen with respect to tPA expression. The expression levels are consistent with the RNA products that are observed; as more of the dicistronic message is spliced an increased amount of message will contain tPA as the first open reading frame resulting in increased tPA expression. A mutation of GT to CA in the splice donor site results in an abundance of DHFR positive colonies which express undetectable levels of tPA, possibly resulting from inefficient utilization of the second AUG. Importantly, Figure 5A also shows that expression levels obtained from one of the dicistronic vectors (with WT ras SD) was about threefold higher than that obtained with the control vector containing a CMV promoter/enhancer driving tPA, SV40

promoter/enhancer controlling DHFR and SV40 polyadenylation signals controlling the expression of tPA and DHFR.

Additionally, the homogeneity of expression in the pools was investigated. Figure 5B shows that all 20 clones generated by the WT ras splice donor site derived dicistronic vectors express detectable levels of tPA while only 4 of 20 clones generated by the control vector express tPA. None of the clones transfected with the non-splicing (ΔGT) vector expressed tPA levels detectable by ELISA. This finding is consistent with previous observations that relatively few clones generated by conventional vectors make useful levels of protein.

Expression of tPA was increased following methotrexate amplification of pools. Figure 5C shows that 2 of the dicistronic vector derived pools (*i.e.* with WT ras and MUTANT ras SD sites) increased in expression markedly (8.4 and 7.7 fold), while the pool generated by the conventional vector increased only slightly (2.8 fold) when each was subjected to 200 nM Mtx. An overall increase of 9 fold was obtained using the best dicistronic (WT ras SD) versus the conventional vector following amplification. Growth of the highest expressing amplified pool in nutrient rich production medium yielded titers of 4.2 μg/ml tPA.

It was shown that manipulation of the splice donor sequence alters the ratio of spliced to full length message and the number of colonies that form in selective medium. It was also shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Surprisingly, it was possible to isolate high expressors which had the efficient WT ras splice donor site by selection for DHFR$^-$ cells despite the efficiency with which the DHFR gene was spliced from the RNA precursors formed in these cells.

EXAMPLE 2

TNFr-IgG production using the dicistronic expression vectors

To prove the general applicability of this approach, a second product was evaluated in the dicistronic vector system containing, as the DNA of interest, an immunoadhesin (TNFr-IgG) capable of binding tumor necrosis factor (TNF) (Ashkenazi *et al.*, Proc. Natl. Acad. Sci. USA, 88:10535-10539 [1991]). The experiments described in Example 1 above were essentially repeated except that the product gene encoded the immunoadhesin TNFr-IgG. Plasmid DNA's that contained a TNFr-IgG cDNA and (a) WT ras, *i.e.* Figure 6 (SEQ ID NO: 2), (b) mutant ras or (c) nonfunctional splice donor site (ΔGT) were introduced into the dp12.CHO cells as discussed for Example 1. See Figure 1C for an illustration of the DNA constructs.

It was discovered that the number of DHFR positive colonies generated by three of these vectors was similar to that seen with the tPA constructs. Expression of TNFr-IgG also paralleled that seen with the tPA constructs (Figure 7A). Amplification of pools from two of the constructs showed a marked increase in expression of immunoadhesin (9.6 and 6.8 fold) (Figure

7B). The best of these amplified pools expressed 9.5 μg/ml when grown in nutrient rich production medium.

Thus, it was again shown that dicistronic expression vectors generate clones that express high levels of recombinant proteins. Furthermore, contrary to expectations, it was discovered that isolation of high product expressing host DHFR$^-$ cells was possible using an efficient splice donor site (*i.e.* the WT ras splice donor site).

EXAMPLE 3

Antibody production using a dicistronic expression vector

The usefulness of this system for antibody expression was evaluated by testing production of an antibody directed against IgE (Presta *et al.*, Journal of Immunology, 151:2623-2632 [1993]). Further, the flexibility of the system with regard to transcription initiation was tested by replacing the CMV promoter/enhancer present in the previous vectors with the promoter/ enhancer derived from the early region of SV40 virus (Griffin, B., Structure and Genomic Organization of SV40 and Polyoma Virus, In J. Tooze [Ed] DNA Tumor Viruses, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The heavy chain of the antibody was inserted downstream of DHFR as described in the earlier tPA and TNFr-IgG constructs. Additionally, a new splice donor site sequence (GAC:GTAAGT) was engineered into the vector which matches the consensus splice donor site more closely than did the splice donor sites present in the vectors tested in Examples 1 and 2. The resultant expression vector is shown in Figures 1D and 9.

It was discovered that this vector produced fewer colonies than the vectors previously tested, and produced predominantly a spliced RNA product. A second vector was constructed to have the light chain of the antibody under control of the SV40 promoter/enhancer and poly-A and the hygromycin B resistance gene under control of the CMV promoter/enhancer and SV40 poly-A. These vectors were linearized at unique HpaI sites downstream of the poly-A signal, mixed at a ratio of light chain vector to heavy chain vector of 10:3 and electroporated into CHO cells using an optimized protocol (as discussed in Examples 1 and 2).

Figure 11 shows the levels of antibody expressed by clones and pools after selection in hygromycin B followed by selection for DHFR expression. All 20 of the clones analyzed expressed high levels of antibody when grown in rich medium and varied from one another by only a factor of four. A pool of antibody producing clones was generated and assayed shortly after it was established. That pool was grown continuously for 6 weeks without a significant decrease in productivity demonstrating that its stability was sufficient to generate gram quantities of protein from its large scale culture.

The pool was subjected to methotrexate amplification at 200nM and 1μM and achieved a greater than 2 fold increase in antibody titer. The 1μM Mtx resistant pool achieved a titer of 41 mg/L when grown under optimal conditions in suspension culture.

The structure of the expressed antibody was examined. Proteins expressed by the 200nM methotrexate resistant pool and by a well characterized expression clone generated by conventional vectors (Presta *et al.* [1993], *supra*) were metabolically labeled with S^{35} cysteine and methionine. In particular, confluent 35mm plates of cells were metabolically labeled with 50μCi each S-35 methionine and S-35 cysteine (Amersham) in serum free cysteine and methionine free F12:DMEM. After one hour, nutrient rich production media was added and labeled proteins were allowed to "chase" into the medium for six more hours. Proteins were run on a 12% SDS/PAGE gel (NOVEX) non-reduced or following reduction with B-mercaptoethanol. Dried gels were exposed to film for 16 hours. CHO control cells were also labeled.

The majority of the antibody protein is secreted with a molecular weight of about 155 kilodaltons, consistent with a properly disulfide-linked antibody molecule with 2 light and 2 heavy chains. Upon reduction the molecular weight shifts to 2 approximately equally abundant proteins of 22.5 and 55 kilodaltons. The protein generated from the pool is indistinguishable from the antibody produced by the well characterized expression clone, with no apparent increase of free heavy or light chain expressed by the pool.

CONCLUSION

The efficient expression system described herein utilizes vectors consisting of promoter/enhancer elements followed by an intron containing the selectable marker coding sequence, followed by the cDNA of interest and a polyadenylation signal.

Several splice donor site sequences were tested for their effect on colony number and expression of the cDNA of interest. A non-functional splice donor site, splice donor sites found in an intron between exons 3 and 4 of mutant (mutant ras) and normal (WT ras) forms of the Harvey Ras gene and another efficient SD site (see Example 3) were used. The vectors were designed to direct expression of dicistronic primary transcripts. Within a transfected cell some of the transcripts remain full length while the remainder are spliced to excise the DHFR coding sequence. When the splice donor site is weakened or destroyed an increase in colony number is observed.

Expression levels show the inverse pattern, with the most efficient splice donor sites generating the highest levels of tPA, TNFr immunoadhesin or anti-IgE V_H.

The homogeneity of expression of clones generated by the ras splice donor site intron DHFR vectors was compared to clones generated from a conventional vector with a separate promoter/enhancer and polyadenylation signal for each DHFR and tPA. The DHFR intron vector gives rise to colonies that are much more homogeneous with regard to expression than those generated by the conventional vector. Non-expressing clones derived from the conventional vector may be the result of breaks in the tPA or

TNFr-IgG domain of the plasmid during integration into the genome or the result of methylation of promoter elements (Busslinger *et al.*, Cell, 34:197-206 [1983]; Watt *et al.*, Genes and Development, 2:1136-1143 [1988]) driving tPA or TNFr-IgG expression. Promoter silencing by methylation or breaks in the DHFR-intron vectors would very likely render them incapable of conferring a DHFR positive phenotype.

It was found that pools generated by the DHFR-intron vectors could be amplified in methotrexate and would increase in expression by a factor of 8.4 (tPA), or 9.8 (TNFr-IgG). Pools from conventional vectors increased by only 2.8 and 3.0 fold for tPA and TNFr-IgG when amplified similarly. Amplified pools resulted in 9 fold higher tPA levels and 15 fold higher TNFr-IgG levels when compared to the conventional vector amplified pools.

Without being limited to any theory, the increase in expression of methotrexate resistant pools derived from the dicistronic vectors is likely due to the transcriptional linkage of DHFR and the product; when cells are selected for increased DHFR expression they consistently over-express product. Conventional approaches lack selectable marker and cDNA expression linkage and therefore methotrexate amplification often generates DHFR overexpression without the concomitant increase in product expression.

A further increase of 4 and 6.3 fold in expression were obtained when amplified tPA and TNFr-IgG pools were transferred from the media used for the selections and amplifications to a nutrient rich production medium.

In Example 3, the expression vector had a splice donor site that more closely matches the consensus splice donor sequence and had the heavy chain of a humanized anti-IgE antibody inserted downstream. This vector was linearized and co-electroporated with a second linearized vector that expresses the hygromycin resistance gene and the light chain of the antibody each under the control of its own promoter/enhancer and poly-A signals. An excess of light chain expression vector over the heavy chain dicistronic expression vector was used to bias in favor of light chain expression. Clones and a pool were generated after hygromycin B and DHFR selections. The clones were found to express relatively consistent, high levels of antibody, as did the pool. The 1μM pool achieved a titer of 41mg/L when grown under optimal conditions in suspension culture.

The anti-IgE antibody was assessed by metabolic labeling followed by SDS/PAGE under reducing and non reducing conditions and found to be indistinguishable from the protein expressed by a highly characterized clonal cell line. Of particular importance is the finding that no free light chain is observed in the pool relative to the clone.

A stable expression system for CHO cells has been developed that produces high levels of recombinant proteins rapidly and with less effort than that required by other expression systems. The vector system generates stable clones that express consistently high levels thereby reducing the number of clones that must be screened to obtain a highly productive clonal line. Alternatively, pools have been used to conveniently generate moderate to high levels of protein. This approach

may be particularly useful when a number of related proteins are to be expressed and compared.

Without being limited to this theory, it is possible the vectors that have very efficient splice donor sites generate very productive clones because so little transcript remains non spliced that only integration events that lead to the generation of high levels of RNA produce enough DHFR protein to give rise to colonies in selective medium. The high level of spliced message from such clones is then translated into abundant amounts of the protein of interest. Pools of clones made concurrently by introducing conventional vectors expressed lower levels of protein, and were unstable with regard to long term expression, and expression could not be appreciably increased when the cells were subjected to methotrexate amplification.

The system developed herein is versatile in that it allows high levels of single and multiple subunit polypeptides to be rapidly generated from clones or pools of stable transfectants. This expression system combines the advantages of transient expression systems (rapid and labor non intensive generation of research amounts of protein) with the concurrent development of highly productive stable production cell lines.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GENENTECH, INC.

(ii) TITLE OF INVENTION: METHOD FOR SELECTING HIGH-EXPRESSING HOST CELLS

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: South San Francisco
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94080

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 5.25 inch, 360 Kb floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: patin (Genentech)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/286740
(B) FILING DATE: 05-AUG-1994

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7360 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA 750

GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT 800

GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA 850

CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC 900

AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA 950

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ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA 1300

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AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA 1400

GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA 1500

CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

GGTCAACTGC ACCTCGGTTC TAAGCTTGGG CTGCAGGTCG CCGTGAATTT 1600

AAGGGACGCT GTGAAGCAAT CATGGATGCA ATGAAGAGAG GGCTCTGCTG 1650

TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT TTCGCCCAGC CAGGAAATCC 1700

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GCTCAGAAGC AACCGGGTGG AATATTGCTG GTGCAACAGT GGCAGGGCAC 1850

AGTGCCACTC AGTGCCTGTC AAAAGTTGCA GCGAGCCAAG GTGTTTCAAC 1900

GGGGGCACCT GCCAGCAGGC CCTGTACTTC TCAGATTTCG TGTGCCAGTG 1950

CCCCGAAGGA TTTGCTGGGA AGTGCTGTGA AATAGATACC AGGGCCACGT 2000

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GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC 2300

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CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG 2650

GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA 2700

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GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT 2800

GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT 2850

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CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT 3400

TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG 3450

GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT 3500

GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA 3550

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GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA 3650

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TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA 3750

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TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC 5500

CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC 5550

GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT 5600

ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC 5650

ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC 5700

ATGAGTGATA ACACTGCGGC CAACTTACTT CTGACAACGA TCGGAGGACC 5750

GAAGGAGCTA ACCGCTTTTT TGCACAACAT GGGGGATCAT GTAACTCGCC 5800

TTGATCGTTG GGAACCGGAG CTGAATGAAG CCATACCAAA CGACGAGCGT 5850

GACACCACGA TGCCAGCAGC AATGGCAACA ACGTTGCGCA AACTATTAAC 5900

TGGCGAACTA CTTACTCTAG CTTCCCGGCA ACAATTAATA GACTGGATGG 5950

AGGCGGATAA AGTTGCAGGA CCACTTCTGC GCTCGGCCCT TCCGGCTGGC 6000

TGGTTTATTG CTGATAAATC TGGAGCCGGT GAGCGTGGGT CTCGCGGTAT 6050

CATTGCAGCA CTGGGGCCAG ATGGTAAGCC CTCCCGTATC GTAGTTATCT 6100

ACACGACGGG GAGTCAGGCA ACTATGGATG AACGAAATAG ACAGATCGCT 6150

GAGATAGGTG CCTCACTGAT TAAGCATTGG TAACTGTCAG ACCAAGTTTA 6200

CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA 6250

TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT 6300

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TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA 6400

AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT 6450

CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT 6500

CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC 6550

CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT 6600

GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA 6650

TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT 6700

TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA 6750

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CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG 6850

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CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG 6950

CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA 7000

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TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA 7250

TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG 7300

GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT 7350

TACGAATTAA 7360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6889 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

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GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC 1450

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CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA 1550

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TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG 2900

CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG 2950

CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC 3000

AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC 3050

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TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA 3250

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GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC 3350

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ATCGCCCTGA TAGACGGTTT TTCGCCCTTT GACGTTGGAG TCCACGTTCT 4250

TTAATAGTGG ACTCTTGTTC CAAACTGGAA CAACACTCAA CCCTATCTCG 4300

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TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT 4900

TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT 4950

GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA 5000

CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA 5050

TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC 5100

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TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT 5900

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AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA 6800

CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT 6850

TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA 6889

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6557 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG 50

GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA 100

GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG 150

TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA 200

GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC 250

CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT 300

TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG 350

AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG 400

CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA 450

GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT 500

TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA 550

AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC 600

CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT 650

TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA 700

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GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA 800

ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA 850

GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT 900

GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT 950

TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG 1000

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TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC 2000

CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT 2050

GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC 2100

TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT 2150

CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA 2200

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CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG 2500

ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG 2550

ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA 2600

GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT 2650

CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC 2700

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AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG 5500

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TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC 5750

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AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG 6000

CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT 6050

GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA 6100

TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA 6150

CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT 6200

TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT 6250

GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC 6300

AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG 6350

CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG 6400

AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA 6450

GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA 6500

TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC 6550

GAATTAA 6557

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7305 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50

TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100

TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150

ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200

TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250

ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300

AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350

TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400

GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450

TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500

AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550

AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600

TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650

CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA 700

TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750

GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA 800

CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850

CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900

ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG 950

TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT 1000

GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG 1050

GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA 1100

GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA 1150

AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC 1200

GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC 1250

GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA 1300

TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC 1350

AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG 1400

TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA 1450

GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC 1500

ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC 1550

CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA 1600

ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG 1650

AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG 1700

CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG 1750

CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG 1800

ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC 1850

CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA 1900

TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA 1950

GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC 2000

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GCCGTCGTTT TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT 4200

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TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG 6900

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GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG 7050

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CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT 7250

GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA 7300

ATTAA 7305

CLAIMS

1. A DNA construct comprising a transcriptional initiation site, a transcriptional termination site, a selectable gene, a product gene provided 3' to the selectable gene, a transcriptional regulatory region regulating transcription of both the selectable gene and the product gene, the selectable gene being positioned within an intron having a splice donor site 5' of the intron, which splice donor site regulates expression of the product gene using the transcriptional regulatory region.

2. The DNA construct of claim 1 wherein the splice donor site comprises an efficient splice donor sequence.

3. The DNA construct of claim 2 wherein the splice donor site comprises a consensus splice donor sequence.

4. The DNA construct of claim 2 wherein the splice donor site comprises the sequence GACGTAAGT.

5. The DNA construct of claim 1 wherein the selectable gene is an amplifiable gene.

6. The DNA construct of claim 5 wherein the amplifiable gene is DHFR.

7. The DNA construct of claim 1 wherein the transcriptional regulatory region comprises a promoter and an enhancer.

8. A vector comprising the DNA construct of claim 1.

9. The vector of claim 8 wherein the selectable gene of the DNA construct is an amplifiable gene.

10. The vector of claim 8 that is capable of replication in a eukaryotic host.

11. A eukaryotic host cell comprising the vector of claim 10.

12. A eukaryotic host cell comprising the DNA construct of claim 5.

13. The host cell of claim 11 wherein the vector is introduced into the host cell by electroporation.

14. A eukaryotic host cell comprising the DNA construct of claim 1 integrated into a chromosome of the host cell.

15. The host cell of claim 14 that is a mammalian cell.

16. A method for producing a product of interest comprising culturing the host cell of claim 11 so as to express the product gene and recovering the product from the host cell culture.

17. The method of claim 16 further comprising recovering the product from the culture medium.

18. The method of claim 16 wherein the selectable gene is an amplifiable gene and the splice donor site comprises an efficient splice donor sequence.

19. A method for producing a product of interest comprising culturing the host cell of claim 12 so as to express the product gene in a selective medium comprising an amplifying agent for sufficient time to allow amplification to occur, and recovering the product.

20. A method for producing eukaryotic cells having multiple copies of a product gene comprising transforming eukaryotic cells with the DNA construct of claim 5, growing the cells in a selective medium comprising an amplifying agent for a sufficient time for amplification to occur, and selecting cells having multiple copies of the product gene.

21. The method of claim 20 further comprising recovering from the selected cells the product of interest.

FIG. 1A

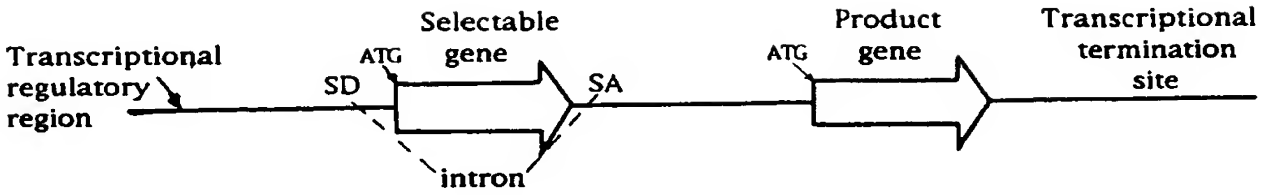
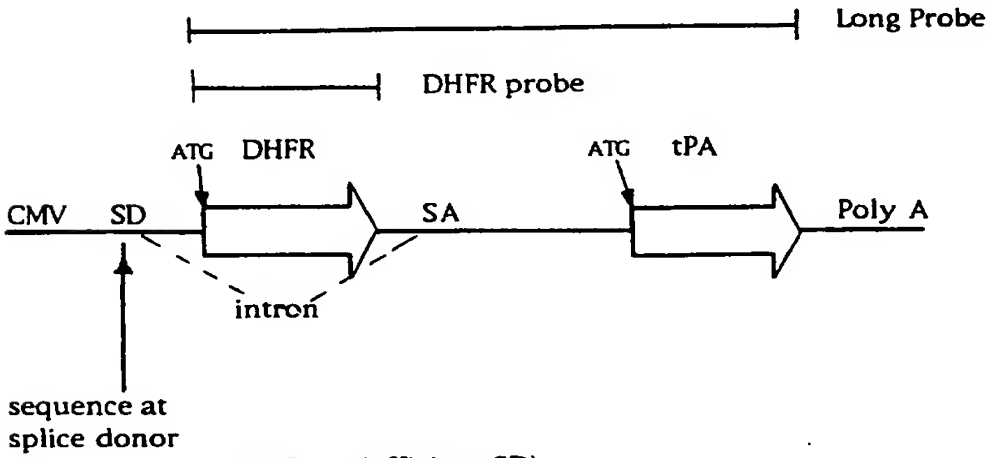
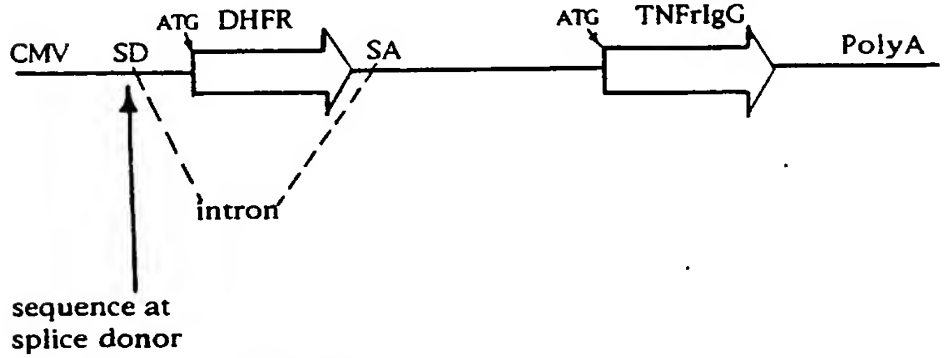


FIG. 1B



a) WT ras (efficient SD)
b) MUTANT ras (less efficient SD)
c) ▲GT (inefficient SD)

FIG. 1C



a) WT ras
b) MUTANT ras
c) ▲GT

FIG. 1D

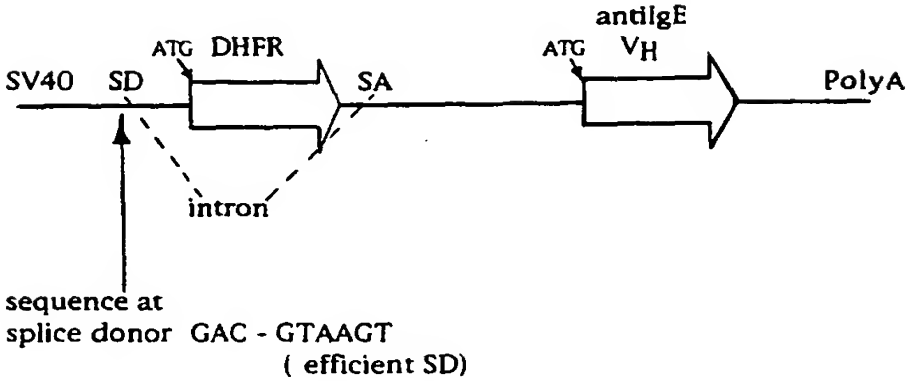


FIG. 2

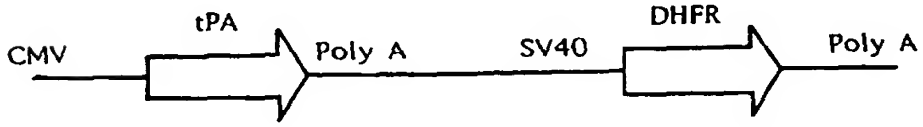


FIG. 3A

```
        aluI
       sstI
       sacI
       hgiJII
       hgiAI/aspHI
       ecl136II
       bsp1286
       bsiHKAI
       bmyI                       rmaI    tru9I
       banII                      maeI    mseI                                                     thaI
     taqI                        speI    aseI/asnI/vspI                        bslI              aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                  scrFI
                  mvaI
                  ecoRII
                  dsaV
               aciI
             bglI bstNI                               maeII
            sau96I                                  hinlI/acyI
            haeIII/palI     aciI                    ahaII/bsaHI
            asuI  apyI[dcm+]            aciI        aatII        maeII            maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

       maeII                                                                                   maeII
      hinlI/acyI                                                                              hinlI/acyI
      ahaII/bsaHI                                rsaI                       rsaI              ahaII/bsaHI
      aatII                             bglI     csp6I           ndeI       csp6I             aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

               scrFI
               mvaI
               ecoRII
           aciI                                                                                  nlaIII
         bglI  dsaV                                                                        styI
        sau96I bstNI            rsaI                                          maeII               ncoI
        haeIII/palI             csp6I                                rsaI    snaBI               dsaI hphI  aciI
        asuI   apyI[dcm+]    bsrI  nlaIII                            csp6I   bsaAI               bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
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FIG. 3B

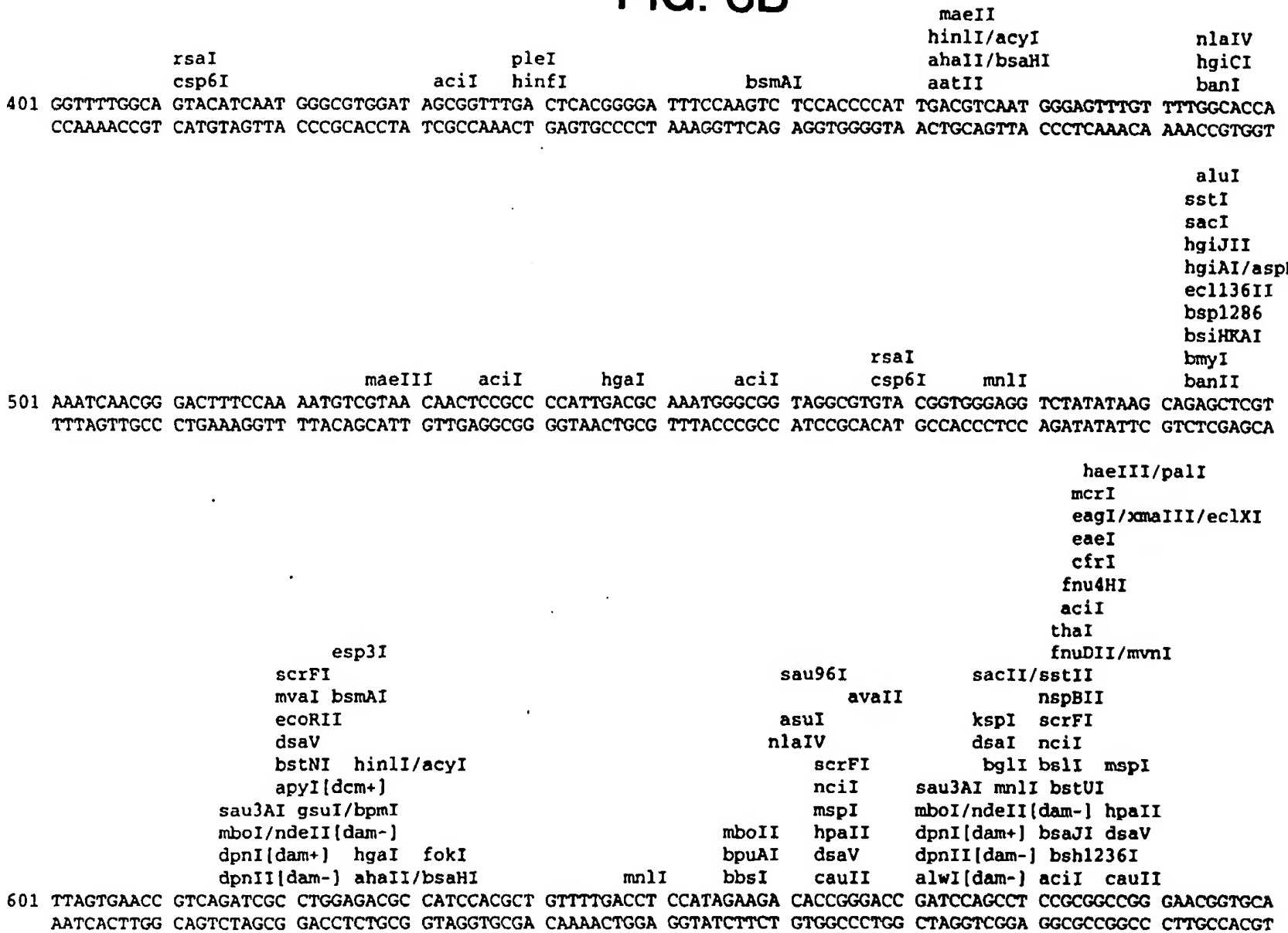


FIG. 3C

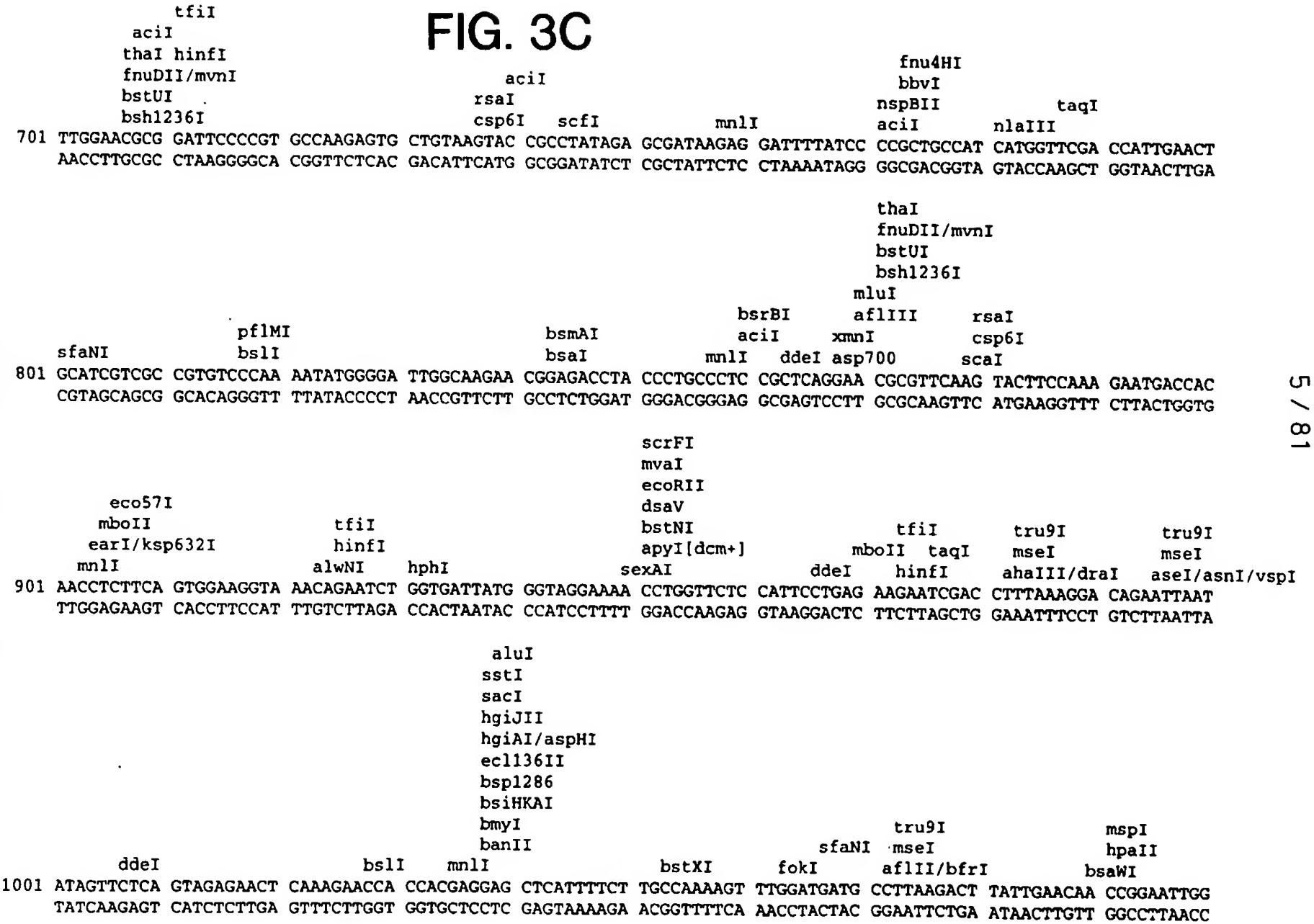


FIG. 3D

```
                                                                                   haeIII/palI
                                                                                  haeI
                                                       scrFI         scrFI                                nlaIII
                                                       mvaI                mvaI                                   sau3AI
                                                       ecoRII        ecoRII                            mboI/ndeII[dam-]
                                                       dsaV   tfiI   dsaV   pleI                       dpnI[dam+]
                                                       bstNI    nlaIII     bstNI    ddeI                          dpnII[dam-]
                accI  nlaIII                mnlI       apyI[dcm+]  hinfI   apyI[dcm+]   hinfI   maeIII  alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                          mnlI
                                                                                     hinlI/acyI      scrFI
                                                                                     ahaII/bsaHI     mvaI
                                                                                  scrFI       ecoRII
                                                                                  mvaI    ecoNI      dsaV
                                                                                  ecoRII     sau96I
                                                                                  dsaV       avaII
                          maeII                                                   bstNI   bslI   asuI   mnlI
                        aflIII                                                    apyI[dcm+]   mnlI  bstNI
          apoI        maeIII                                  mnlI               bsaJI hgaI    ddeI  apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                            ppu10I
                                                            sfaNI                           nsiI/avaIII
          sfaNI                   accI     mboII         mboII                        mnlI    aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                       fnu4HI
         nlaIII                                        aciI
        styI                                          thaI
        ncoI                                          fnuDII/mvnI tru9I
        dsaI                        styI              bstUI      mseI                                  maeIII
        bsaJI                       bsaJI             bsh1236I  aseI/asnI/vspI                         hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 3E

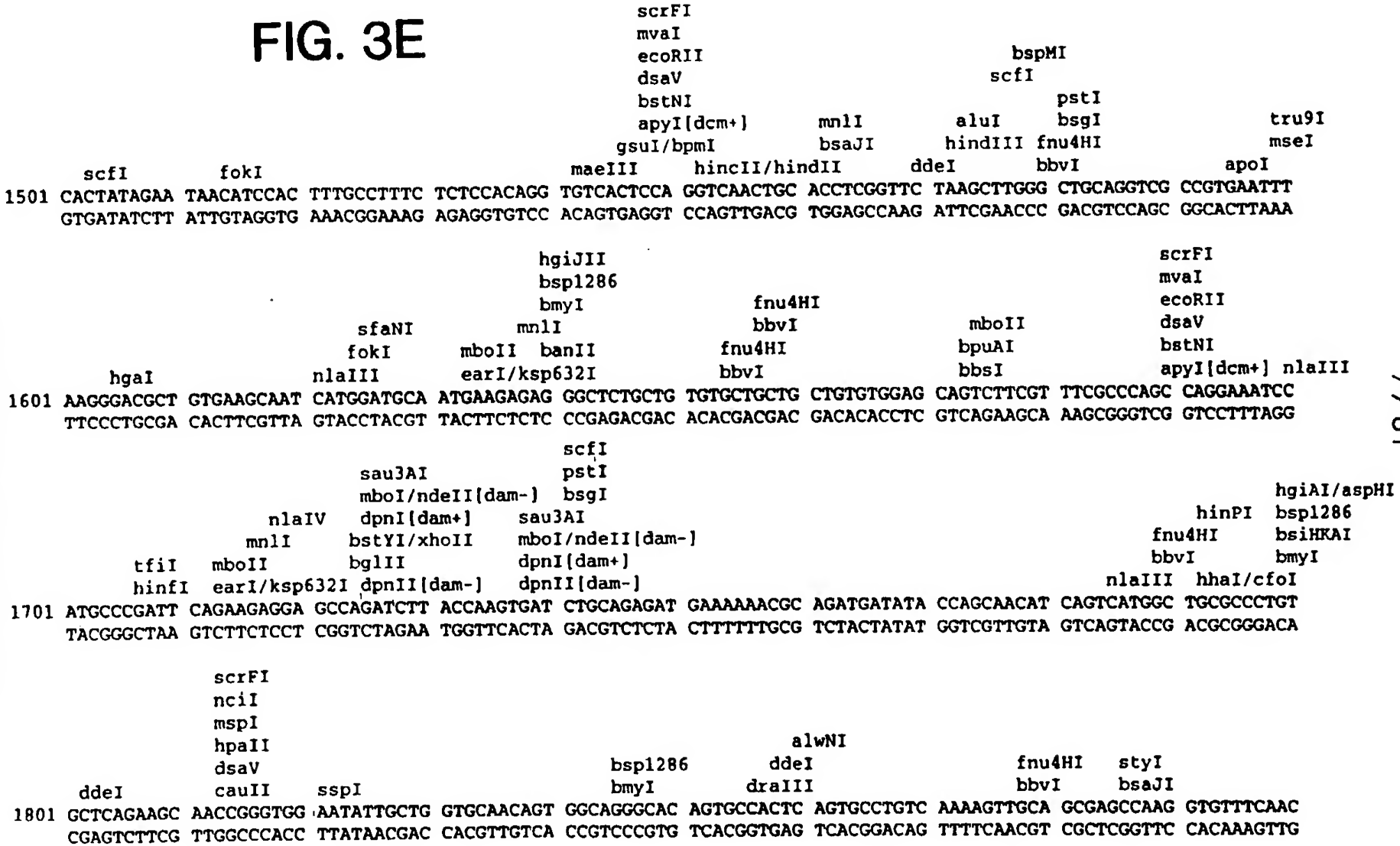


FIG. 3F

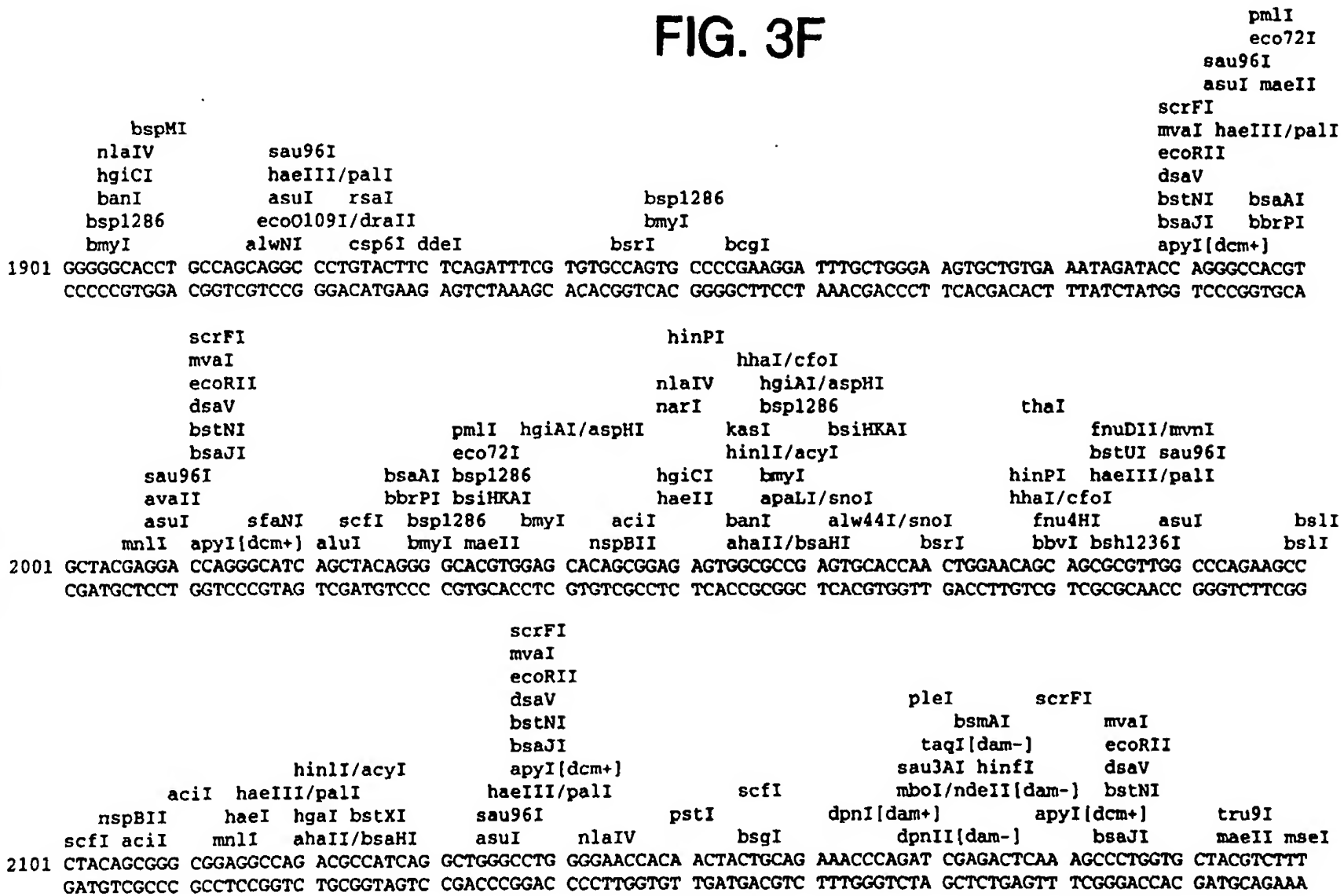


FIG. 3G

```
                                  fnu4HI
                                  bbvI
                 aluI           scfI
              rsaI              pstI                     mnlI        maeIII                                   dsaI
       aciI   csp6I ddeI        bsgI                   ddeI        alwNI                                      bsaJI
2201 AAGGCGGGGA AGTACAGCTC AGAGTTCTGC AGCACCCCTG CCTGCTCTGA GGGAAACAGT GACTGCTACT TTGGGAATGG GTCAGCCTAC CGTGGCACGC
     TTCCGCCCCT TCATGTCGAG TCTCAAGACG TCGTGGGGAC GGACGAGACT CCCTTTGTCA CTGACGATGA AACCCTTACC CAGTCGGATG GCACCGTGCG
                                                                                                     scrFI
                                                                                                     pflMI
                                                                                                     mvaI
                                                                                                     ecoRII
                                                                                                     dsaV
                                                         sau3AI                                      bstNI
                                                         mboI/ndeII[dam-]                            bslI
                                                         dpnI[dam+]                                  apyI[dcm+] haeIII/palI
              pleI  nlaIV                   ecoRI        dpnII[dam-]                              bsp1286       sau96I
         hphI hinfI hgiCI               dsaI apoI        alwI[dam-]                               bmyI alwNI    asuI
       mnlI  bcgI   banI mnlI     mnlI bsaJI          nlaIII                                   bsrI  bsaJI   bsrI
2301 ACAGCCTCAC CGAGTCGGGT GCCTCCTGCC TCCCGTGGAA TTCCATGATC CTGATAGGCA AGGTTTACAC AGCACAGAAC CCCAGTGCCC AGGCACTGGG
     TGTCGGAGTG GCTCAGCCCA CGGAGGACGG AGGGCACCTT AAGGTACTAG GACTATCCGT TCCAAATGTG TCGTGTCTTG GGGTCACGGG TCCGTGACCC
                                                              nlaIV
                                                              hgiCI
                                                              banI  maeII
                                                          scrFI
     scrFI                                        mvaI       pmlI
     mvaI                                                 ecoRII
     ecoRII                                       dsaV
     dsaV                tfiI                     bstNI      eco72I
     bstNI                     hinfI              pflMI      bsaAI                                rsaI
     bsaJI                  mspI              sfaNI       apyI[dcm+]       mboII                       csp6I      bsp1286
     apyI[dcm+]             hpaII            fokI  bslI bsaJI       bbrPI eco57I  aciI        maeII  scaI       bmyI
2401 CCTGGGCAAA CATAATTACT GCCGGAATCC TGATGGGGAT GCCAAGCCCT GGTGCCACGT GCTGAAGAAC CGCAGGCTGA CGTGGGAGTA CTGTGATGTG
     GGACCCGTTT GTATTAATGA CGGCCTTAGG ACTACCCCTA CGGTTCGGGA CCACGGTGCA CGACTTCTTG GCGTCCGACT GCACCCTCAT GACACTACAC
                                                                                                     scrFI
                                                                          mboII                      mvaI
                                                                         earI/ksp632I                ecoRII
                                                                      hgiJII                         dsaV
                                                                      bsp1286                        bstNI
                fnu4HI  bsmAI                                         bmyI                           apyI[dcm+]
                aciI  ddeI     rsaI          ddeI                     banII                     bslI bsaJI     fnu4HI
      mnlI   bspMI haeIII/palI csp6I       mnlI         sfaNI      mnlI sapI              mnlI   bslI        bbvI
2501 CCCTCCTGCT CCACCTGCGG CCTGAGACAG TACAGCCAGC CTCAGTTTCG CATCAAAGGA GGGCTCTTCG CCGACATCGC CTCCCACCCC TGGCAGGCTG
     GGGAGGACGA GGTGGACGCC GGACTCTGTC ATGTCGGTCG GAGTCAAAGC GTAGTTTCCT CCCGAGAAGC GGCTGTAGCG GAGGGTGGGG ACCGTCCGAC
```

FIG. 3H

```
                                      mspI
                                      hpaII
                                      bslI
                                     scrFI                                                                                          scrFI
                                     nciI       nlaIV                                                                               mvaI
                                     dsaV      aciI                                                                                 ecoRII
                                     dsaV      aciI                            aluI        tfiI        aciI                         dsaV
         bslI        mnlI            cauII   bsrBI     bslI aciI               alwNI       hinfI      fnu4HI                        bstNI
                                                                                                                                    apyI[dcm+]
2601 CCATCTTTGC CAAGCACAGG AGGTCGCCCG GAGAGCGGTT CCTGTGCGGG GGCATACTCA TCAGCTCCTG CTGGATTCTC TCTGCCGCCC ACTGCTTCCA
     GGTAGAAACG GTTCGTGTCC TCCAGCGGGC CTCTCGCCAA GGACACGCCC CCGTATGAGT AGTCGAGGAC GACCTAAGAG AGACGGCGGG TGACGAAGGT

                                                                       scrFI
                                                                       mvaI
                                                                       ecoRII
                                                                       dsaV
                                                                       bstNI
                                                                       apyI[dcm+]
                                                            scrFI     bslI
                                           sau3AI           nciI      bsaJI
                                           mboI/ndeII[dam-] mspI   sau96I
                                           dpnI[dam+]       hpaII  nlaIV
                                           dpnII[dam-]      dsaV   avaII          mnlI
    mnlI       aciI      bslI         hphI[dam-]            cauII  asuI        mnlI         apoI        taqI
2701 GGAGAGGTTT CCGCCCCACC ACCTGACGGT GATCTTGGGC AGAACATACC GGGTGGTCCC TGGCGAGGAG GAGCAGAAAT TTGAAGTCGA AAAATACATT
     CCTCTCCAAA GGCGGGGTGG TGGACTGCCA CTAGAACCCG TCTTGTATGG CCCACCAGGG ACCGCTCCTC CTCGTCTTTA AACTTCAGCT TTTTATGTAA

                                                            scfI                                  scrFI
                                                            pstI                                  mvaI
                                                           fnu4HI                                 ecoRII
                                                           bbvI pvuII                             dsaV
                                                        fnu4HI fnu4HI                             bstNI              aciI
                                                        bbvI   bbvI                          bsp1286      fnu4HI   sau96I
           ecoRI                                      hinPI bsgI aluI          tfiI      nspBII  bsaJI    bbvI     avaII
           apoI taqI                                  hhaI/cfoI nspBII         hinfI     aciI bmyI apyI[dcm+]      asuI
2801 GTCCATAAGG AATTCGATGA TGACACTTAC GACAATGACA TTGCGCTGCT GCAGCTGAAA TCGGATTCGT CCCGCTGTGC CCAGGAGAGC AGCGTGGTCC
     CAGGTATTCC TTAAGCTACT ACTGTGAATG CTGTTACTGT AACGCGACGA CGTCGACTTT AGCCTAAGCA GGGCGACACG GGTCCTCTCG TCGCACCAGG
```

FIG. 3I

```
                                                               aluI
                                                              sstI
                                   pvuII                      sacI
                      mspI      scfI                          hgiJII
                      hpaII     pstI  fnu4HI                  hgiAI/aspHI
                     scrFI    bspMI   bbvI                    ecl136II                   haeIII/palI
                     nciI   sau96I nspBII                     bsp1286                   stuI
                     dsaV   avaII fnu4HI      bsrI            bsiHKAI                   haeI
                     cauII  asuI  bbvI    mspI                bmyI    mspI             mnlI
      draIII   bslI bsaJI aciI  bsgI aluI hpaII               banII   hpaII         nlaIII      bsmAI
2901 GCACTGTGTG CCTTCCCCCG GCGGACCTGC AGCTGCCGGA CTGGACGGAG TGTGAGCTCT CCGGCTACGG CAAGCATGAG GCCTTGTCTC CTTTCTATTC
     CGTGACACAC GGAAGGGGGC CGCCTGGACG TCGACGGCCT GACCTGCCTC ACACTCGAGA GGCCGATGCC GTTCGTACTC CGGAACAGAG GAAAGATAAG

                                                    fnu4HI
                                                  nspBII
        fnu4HI                                    aciI                                          nlaIII
        aciI                         rsaI fokI   fnu4HI                    tru9I       hphI      nspI
      bsrBI eco57I mnlI  nlaIII      csp6I    alwNI bbvI                   mseI       maeIII     nspHI        gsuI/bpmI
3001 GGAGCGGCTG AAGGAGGCTC ATGTCAGACT GTACCCATCC AGCCGCTGCA CATCACAACA TTTACTTAAC AGAACAGTCA CCGACAACAT GCTGTGTGCT
     CCTCGCCGAC TTCCTCCGAG TACAGTCTGA CATGGGTAGG TCGGCGACGT GTAGTGTTGT AAATGAATTG TCTTGTCAGT GGCTGTTGTA CGACACACGA

                         sau96I
                         nlaIV
                         haeIII/palI
                         asuI
                        sau96I
                        nlaIV scrFI                                     scrFI
                        hgiJII                                                mvaI
                        eco0109I/draII                 scrFI            ecoRII
                        bsp1286                        mvaI                   dsaV
                        bsp120I                        ecoRII           bstNI
                        bmyI  mvaI                     dsaV        sau96I
                        banII ecoRII             bstNI             nlaIV                       aciI
                      aciI    dsaV               bsaJI             haeIII/palI                fnu4HI
                   fnu4HI     bstNI           hinlI/acyI       tfiI      asuI apyI[dcm+]           haeIII/palI
                   aciI asuI  apyI[dcm+]      hgaI     apyI[dcm+]       eco0109I/draII            eaeI
      bsmAI     bsrBI  apaI bsaJI            ahaII/bsaHI       hinfI   mnlI  bsaJI                cfrI  nlaIII
3101 GGAGACACTC GGAGCGGCGG GCCCCAGGCA AACTTGCACG ACGCCTGCCA GGGCGATTCG GGAGGCCCCC TGGTGTGTCT GAACGATGGC CGCATGACTT
     CCTCTGTGAG CCTCGCCGCC CGGGGTCCGT TTGAACGTGC TGCGGACGGT CCCGCTAAGC CCTCCGGGGG ACCACACAGA CTTGCTACCG GCGTACTGAA
```

FIG. 3J

```
                                                        scrFI
                                                        nciI
                             scrFI                mspI
                             mvaI                       hpaII
                             ecoRII               dsaV
                             dsaV                 cauII
                             bstNI                     xmaI/pspAI
                             bsaJI                     smaI
                             apyI[dcm+]                scrFI
                        nlaIV                          nciI
                        ecoO109I/draII           dsaV
                    aluI sau96I                  cauII     rsaI  styI maeIII                     tfiI        nspI
                   pvuII asuI                          bsaJI     csp6I bsaJI                 rmaI     hinfI       nspHI
           sfaNI   nspBII  haeIII/palI           fokI  avaI     bsp1407I   bstEII            maeI bsrI   maeIII nlaIII
3201 TGGTGGGCAT CATCAGCTGG GGCCTGGGCT GTGGACAGAA GGATGTCCCG GGTGTGTACA CCAAGGTTAC CAACTACCTA GACTGGATTC GTGACAACAT
     ACCACCCGTA GTAGTCGACC CCGGACCCGA CACCTGTCTT CCTACAGGGC CCACACATGT GGTTCCAATG GTTGATGGAT CTGACCTAAG CACTGTTGTA

                                                            mboII
                scrFI                            aciI earI/ksp632I
                mvaI                               sau3AI mnlI
                ecoRII                       mboI/ndeII[dam-]
                dsaV                         dpnI[dam+]           mboII
                bstNI            mnlI              dpnII[dam-]    eco57I
         bslI   apyI[dcm+]   pleI                  alwI[dam-]    mboII  bpuAI           hinPI
      mcrI maeIII            hinfI               bstYI/xhoII  mboII     bbsI            hhaI/cfoI     scfI
3301 GCGACCGTGA CCAGGAACAC CCGACTCCTC AAAAGCAAAT GAGATCCCGC CTCTTCTTCT TCAGAAGACA CTGCAAAGGC GCAGTGCTTC TCTACAGACT
     CGCTGGCACT GGTCCTTGTG GGCTGAGGAG TTTTCGTTTA CTCTAGGGCG GAGAAGAAGA AGTCTTCTGT GACGTTTCCG CGTCACGAAG AGATGTCTGA

                                        bsmAI               mboII
      gsuI/bpmI        aciI     aciI    bsaI  scfI     mnlI earI/ksp632I                                   tth111I/aspI
3401 TCTCCAGACC CACCACACCG CAGAAGCGGG ACGAGACCCT ACAGGAGAGG GAAGAGTGCA TTTTCCCAGA TACTTCCCAT TTTGGAAGTT TTCAGGACTT
     AGAGGTCTGG GTGGTGTGGC GTCTTCGCCC TGCTCTGGGA TGTCCTCTCC CTTCTCACGT AAAAGGGTCT ATGAAGGGTA AAACCTTCAA AAGTCCTGAA

                                                                                    scrFI
                                                                 scrFI       mvaI
                                                                 mvaI bsmI          ecoRII
                                                                 ecoRII      dsaV
                                                                 dsaV        bstNI
                                      mboII                      bstNI              bsaJI
                                      bpuAI   bsmI      rmaI    gsuI/bpmI  mnlI    bsaJI                ecoRI   taqI
                                      bbsI nlaIII       maeI mnlI apyI[dcm+]  mnlI apyI[dcm+]           apoI   claI/bsp106
3501 GGTCTGATTT CAGGATACTC TGTCAGATGG GAAGACATGA ATGCACACTA GCCTCTCCAG GAATGCCTCC TCCCTGGGCA GAAGTGGGGG GAATTCAATC
     CCAGACTAAA GTCCTATGAG ACAGTCTACC CTTCTGTACT TACGTGTGAT CGGAGAGGTC CTTACGGAGG AGGGACCCGT CTTCACCCCC CTTAAGTTAG
```

FIG. 3K

```
                styI
               aciI
              fnu4HI   sau96I
              bglI nlaIII
             sfiI ncoI haeIII/palI
             haeIII/palI                    aluI
            eaeI  dsaI asuI               fnu4HI
            cfrI  bsaJI                   bbvI          maeIII              sfaNI  apoI                                  bsmI
3601 GATGGCCGCC ATGGCCCAAC TTGTTTATTG CAGCTTATAA TGGTTACAAA TAAAGCAATA GCATCACAAA TTTCACAAAT AAAGCATTTT TTTCACTGCA
     CTACCGGCGG TACCGGGTTG AACAAATAAC GTCGAATATT ACCAATGTTT ATTTCGTTAT CGTAGTGTTT AAAGTGTTTA TTTCGTAAAA AAAGTGACGT

                                                          sau3AI
                                                          mboI/ndeII[dam-]
                                                          dpnI[dam+]
                                                          dpnII[dam-]
                                                         pvuI/bspCI
                                                         mcrI
                                                        taqI[dam-]  tru9I
                                                       claI/bsp106[dam-]              styI
                                                      sau3AI          mseI       fnu4HI    haeI
                                                      mboI/ndeII[dam-]           bbvI  ncoI
                                                      dpnI[dam+] xmnI        hinPI    dsaI haeIII/palI
            rmaI                                      dpnII[dam-]   aseI/asnI/vspI    bsaJI
            maeI                                 nlaIII alwI[dam-]  asp700      hhaI/cfoI nlaIII          mnlI
3701 TTCTAGTTGT GGTTTGTCCA AACTCATCAA TGTATCTTAT CATGTCTGGA TCGATCGGGA ATTAATTCGG CGCAGCACCA TGGCCTGAAA TAACCTCTGA
     AAGATCAACA CCAAACAGGT TTGAGTAGTT ACATAGAATA GTACAGACCT AGCTAGCCCT TAATTAAGCC GCGTCGTGGT ACCGGACTTT ATTGGAGACT

                          rsaI                                                                    nlaIV
                          csp6I                                                                 scrFI
                         nlaIV                                                                  mvaI
                         kpnI                                                                   ecoRII
                         hgiCI                                                                  dsaV
                         banI                              aluI                                 bstNI
                         asp718     mnlI                   pvuII                                apyI[dcm+]
            mnlI         acc65I   ddeI aciI               nspBII                               bsaJI
3801 AAGAGGAACT TGGTTAGGTA CCTTCTGAGG CGGAAAGAAC CAGCTGTGGA ATGTGTGTCA GTTAGGGTGT GGAAAGTCCC CAGGCTCCCC AGCAGGCAGA
     TTCTCCTTGA ACCAATCCAT GGAAGACTCC GCCTTTCTTG GTCGACACCT TACACACAGT CAATCCCACA CCTTTCAGGG GTCCGAGGGG TCGTCCGTCT
```

FIG. 3L

```
                                                              nlaIV
           sfaNI                  scrFI        scrFI                                 sfaNI
          ppu10I                  mvaI               mvaI                                      ppu10I
          nsiI/avaIII             ecoRII       ecoRII                               nsiI/avaIII
         nlaIII                   dsaV         dsaV                                nlaIII
        sphI                      bstNI              bstNI                                   sphI
        nspI                      apyI[dcm+]         apyI[dcm+]                              nspI
        nspHI                    sexAI              bsaJI                                    nspHI
3901 AGTATGCAAA GCATGCATCT CAATTAGTCA GCAACCAGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA GAAGTATGCA AAGCATGCAT CTCAATTAGT
     TCATACGTTT CGTACGTAGA GTTAATCAGT CGTTGGTCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT CTTCATACGT TTCGTACGTA GAGTTAATCA

                                                                                     nlaIII
                                                                                    styI
                                                                                    ncoI
                                     aciI                                     bslI  dsaI
           aciI          aciI  fokI              aciI bsrI  aciI              aciI  bsaJI
4001 CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGCC CCTAACTCCG CCCAGTTCCG CCCATTCTCC GCCCCATGGC TGACTAATTT TTTTTATTTA
     GTCGTTGGTA TCAGGGCGGG GATTGAGGCG GGTAGGGCGG GGATTGAGGC GGGTCAAGGC GGGTAAGAGG CGGGGTACCG ACTGATTAAA AAAAATAAAT

                                                                             rmaI
                                                                            styI
           aciI                                                             bsaJI
          fnu4HI                                                            blnI
         sfiI                                                               avrII
         haeIII/palI                                                      haeIII/palI                tru9I
    bsaJI bglI           ddeI                                            stuI                       hpaI
  haeIII/palI mnlI haeIII/palI                        mnlI               haeI.                      hincII/hindII
mnlI    mnlI   bsaJI  mnlI  aluI                 mnlI                   mnlI maeI              aluI mseI  aluI
4101 TGCAGAGGCC GAGGCCGCCT CGGCCTCTGA GCTATTCCAG AAGTAGTGAG GAGGCTTTTT TGGAGGCCTA GGCTTTTGCA AAAAGCTGTT AACAGCTTGG
     ACGTCTCCGG CTCCGGCGGA GCCGGAGACT CGATAAGGTC TTCATCACTC CTCCGAAAAA ACCTCCGGAT CCGAAAACGT TTTTCGACAA TTGTCGAACC

                                       scrFI
                                       mvaI
                                       ecoRII
  haeIII/palI                          dsaV
 eaeI                                  bstNI                                                     aluI
 cfrI                        bsrI      apyI[dcm+]       tru9I       fnu4HI                       pvuII
bsrI                 maeII maeIII     bsaJI   maeIII    mseI        bbvI   fokI                  nspBII
4201 CACTGGCCGT CGTTTTACAA CGTCGTGACT GGGAAAACCC TGGCGTTACC CAACTTAATC GCCTTGCAGC ACATCCCCCC TTCGCCAGCT GGCGTAATAG
     GTGACCGGCA GCAAAATGTT GCAGCACTGA CCCTTTTGGG ACCGCAATGG GTTGAATTAG CGGAACGTCG TGTAGGGGGG AAGCGGTCGA CCGCATTATC
```

FIG. 3M

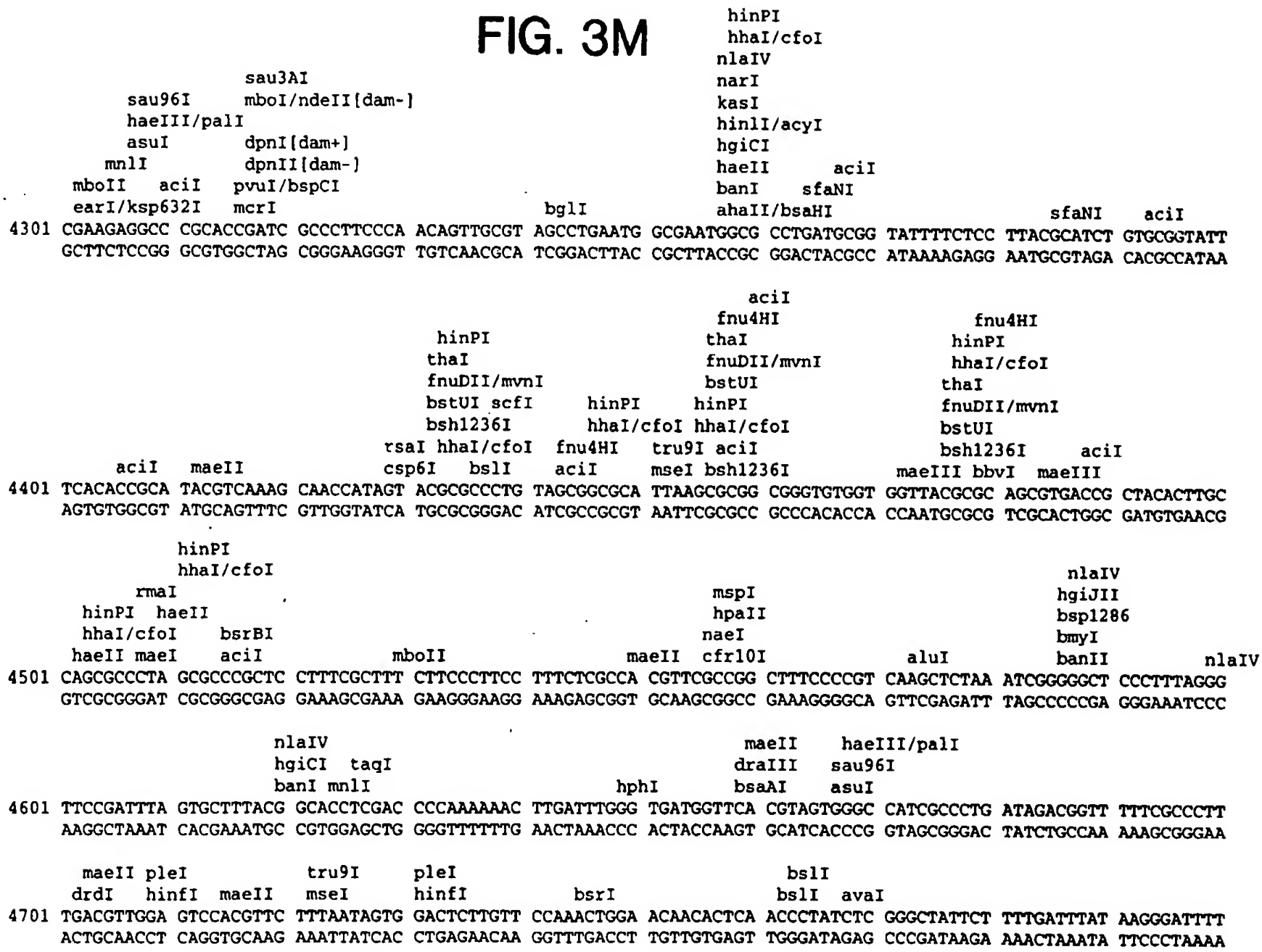


FIG. 3N

```
                                                                                                           hgiAI/aspHI
                                                             thaI                                          bsp1286
                                                             fnuDII/mvnI              maeII                bsiHKAI
                    tru9I                               tru9I   apoI tru9I           psp1406I              bmyI   ddeI
                    mseI                tru9I           mseI bstUI   mseI           tru9I                  apaLI/snoI
          haeIII/palI           aluI    mseI     apoI        bsh1236I        sspI mseI                     alw44I/snoI
4801 GCCGATTTCG GCCTATTGGT TAAAAAATGA GCTGATTTAA CAAAAATTTA ACGCGAATTT TAACAAAATA TTAACGTTTA CAATTTTATG GTGCACTCTC
     CGGCTAAAGC CGGATAACCA ATTTTTTACT CGACTAAATT GTTTTTAAAT TGCGCTTAAA ATTGTTTTAT AATTGCAAAT GTTAAAATAC CACGTGAGAG

                                                                                                                  hinPI
                                                                                                                  hhaI/cfoI
                                                                                                                 thaI
                                                                            hinPI                                fnuDII/mvnI
                    aciI                                        maeIII    fnu4HI                                 bstUI
     rsaI          fnu4HI    tru9I                         maeII bsrI  nlaIII hhaI/cfoI                   nspBII bsh1236I
     csp6I       sfaNI      mseI         aciI             bsaAI tth111I/aspI bbvI                aciI     aciI hgaI
4901 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAACTCC GCTATCGCTA CGTGACTGGG TCATGGCTGC GCCCCGACAC CCGCCAACAC CCGCTGACGC
     TCATGTTAGA CGAGACTACG GCGTATCAAT TCGGTTGAGG CGATAGCGAT GCACTGACCC AGTACCGACG CGGGGCTGTG GGCGGTTGTG GGCGACTGCG

                                                                   scrFI
                               sfaNI                               nciI
                            mspI                                   mspI
                            hpaII                                  hpaII     nspI
                           scrFI                                   dsaV      nspHI
                           nciI                                esp3I      fnu4HI
                           dsaV fokI                     maeIII bsmAI     bbvI
         drdI              cauII   aciI            aluI      bslI  cauII aluI nlaIII   mnlI    hphI      hphI
5001 GCCCTGACGG GCTTGTCTGC TCCCGGCATC CGCTTACAGA CAAGCTGTGA CCGTCTCCGG GAGCTGCATG TGTCAGAGGT TTTCACCGTC ATCACCGAAA
     CGGGACTGCC CGAACAGACG AGGGCCGTAG GCGAATGTCT GTTCGACACT GGCAGAGGCC CTCGACGTAC ACAGTCTCCA AAAGTGGCAG TAGTGGCTTT

       thaI
       fnuDII/mvnI
       bstUI
       bsh1236I
      hinPI
      hhaI/cfoI                             mnlI
     thaI mnlI                            haeIII/palI                                                   hinlI/acyI
     fnuDII/mvnI          mboII          sau96I                                   nlaIII                ahaII/bsaHI
     bstUI                bpuAI          asuI                                tru9I  rcaI                aatII
     bsh1236I             bbsI          eco0109I/draII                       mseI   bspHI           ddeI maeII
5101 CGCGCGAGGC AGTATTCTTG AAGACGAAAG GGCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA TAATAATGGT TTCTTAGACG TCAGGTGGCA
     GCGCGCTCCG TCATAAGAAC TTCTGCTTTC CCGGAGCACT ATGCGGATAA AAATATCCAA TTACAGTACT ATTATTACCA AAGAATCTGC AGTCCACCGT
```

FIG. 3O

```
                          nlaIV
                         aciI
                        thaI
                        fnuDII/mvnI
                        bstUI                                                    rcaI
                        bsh1236I                                                 bspHI
                       hinPI                                                  bsrBI    bsmAI
                       hhaI/cfoI                                              aciI  nlaIII
5201 CTTTTCGGGG AAATGTGCGC GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT CATGAGACAA TAACCCTGAT AAATGCTTCA
     GAAAAGCCCC TTTACACGCG CCTTGGGGAT AAACAAATAA AAAGATTTAT GTAAGTTTAT ACATAGGCGA GTACTCTGTT ATTGGGACTA TTTACGAAGT

                      mboII                                                    fnu4HI
       sspI           earI/ksp632I                                             aciI                              hphI
5301 ATAATATTGA AAAAGGAAGA GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA TTTTGCCTTC CTGTTTTTGC TCACCCAGAA
     TATTATAACT TTTTCCTTCT CATACTCATA AGTTGTAAAG GCACAGCGGG AATAAGGGAA AAAACGCCGT AAAACGGAAG GACAAAAACG AGTGGGTCTT

                                        hgiAI/aspHI
                                        bsp1286
                                sau3AI  bsiHKAI                          sau3AI   nspBII    sau3AI
                                mboI/ndeII[dam-]                         mboI/ndeII[dam-]   mboI/ndeII[dam-]
                                dpnI[dam+] bmyI                          dpnI[dam+]         dpnI[dam+]
                                dpnII[dam-]                             bstYI/xhoII         dpnII[dam-]
                             eco57I          apaLI/snoI                bsrI dpnII[dam-]      alwI[dam-]
       hphI           sfaNI  mboII[dam-]     alw44I/snoI  maeIII taqI    alwI[dam-]  aciI  bstYI/xhoII
5401 ACGCTGGTGA AAGTAAAAGA TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA ACAGCGGTAA GATCCTTGAG AGTTTTCGCC
     TGCGACCACT TTCATTTTCT ACGACTTCTA GTCAACCCAC GTGCTCACCC AATGTAGCTT GACCTAGAGT TGTCGCCATT CTAGGAACTC TCAAAAGCGG

                                                                              scrFI
                                                    aciI                      nciI
                                                   thaI                       mspI
         maeII                                     fnuDII/mvnI                hpaII
        psp1406I          hgiAI/aspHI              bstUI                      dsaV
       xmnI               bsp1286  tru9I           bsh1236I                hinlI/acyI
       asp700             bsiHKAI  mseI           hinPI                   hgaI cauII                    aciI
     mboII                bmyI   ahaIII/draI      hhaI/cfoI               ahaII/bsaHI     bcgI  mcrI  fnu4HI
5501 CCGAAGAACG TTTTCCAATG ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTGATGA CGCCGGGCAA GAGCAACTCG GTCGCCGCAT
     GGCTTCTTGC AAAAGGTTAC TACTCGTGAA AATTTCAAGA CGATACACCG CGCCATAATA GGGCACTACT GCGGCCCGTT CTCGTTGAGC CAGCGGCGTA

                                rsaI
                                csp6I   bsrI                                                    fnu4HI
          ddeI                 scaI  hphI  maeIII      sfaNI     fokI   nlaIII                  bbvI        nlaIII
5601 ACACTATTCT CAGAATGACT TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA GTAAGAGAAT TATGCAGTGC TGCCATAACC
     TGTGATAAGA GTCTTACTGA ACCAACTCAT GAGTGGTCAG TGTCTTTTCG TAGAATGCCT ACCGTACTGT CATTCTCTTA ATACGTCACG ACGGTATTGG
```

FIG. 3P

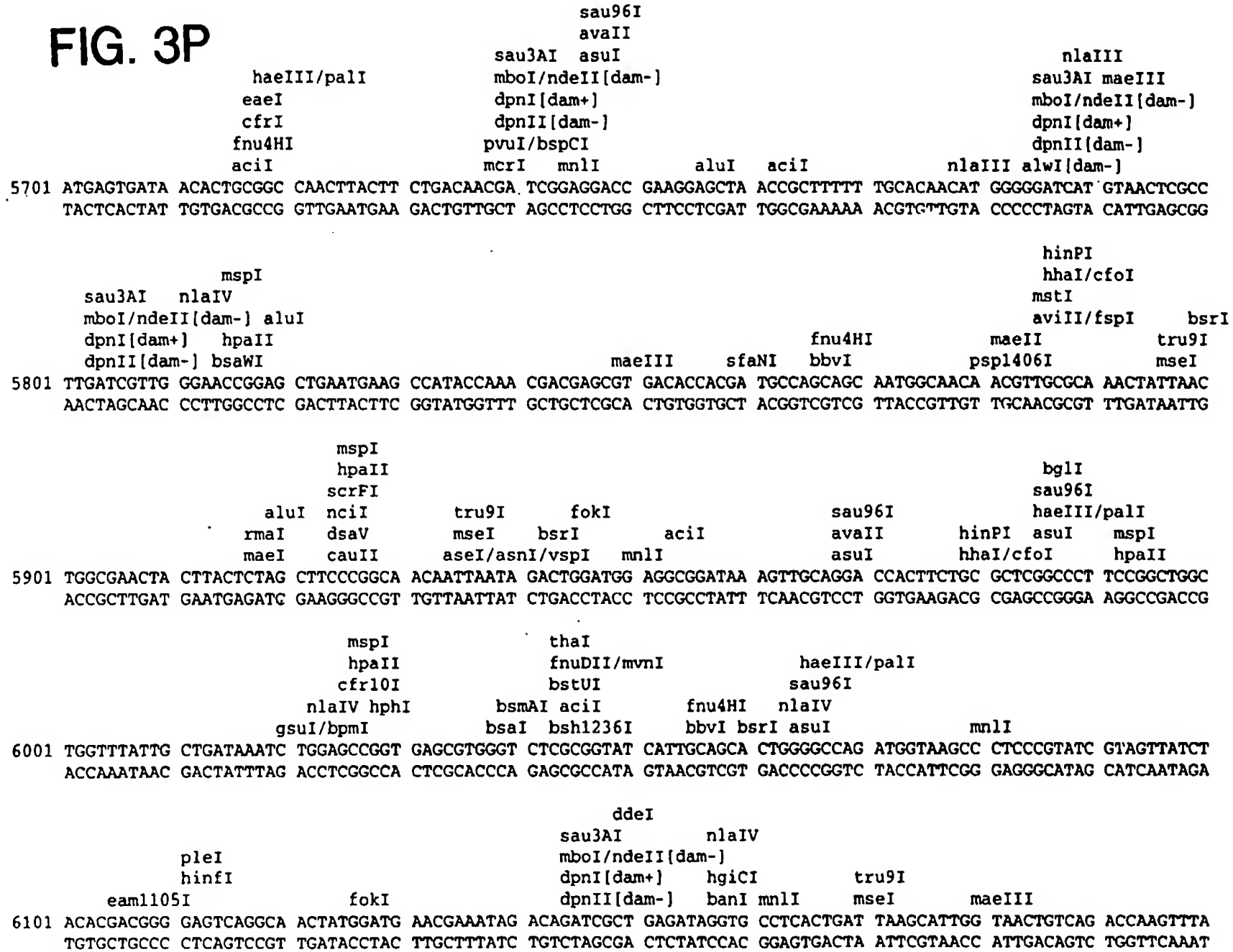


FIG. 3Q

```
                                                rmaI       sau3AI
                                            sau3AI hphI    mboI/ndeII[dam-]
                                            mboI/ndeII[dam-]
                                            dpnI[dam+]     dpnI[dam+]
                                            dpnII[dam-]    dpnII[dam-]
                    tru9I               tru9I bstYI/xhoII  alwI[dam-]          nlaIII              maeII
                    mseI          tru9I mseI  alwI[dam-]  bstYI/xhoII          rcaI             tru9I
                  ahaIII/draI     mseI ahaIII/draI maeI  mboII[dam-]           bspHI            mseI
6201 CTCATATATA CTTTAGATTG ATTTAAAACT TCATTTTTAA TTTAAAAGGA TCTAGGTGAA GATCCTTTTT GATAATCTCA TGACCAAAAT CCCTTAACGT
     GAGTATATAT GAAATCTAAC TAAATTTTGA AGTAAAAATT AAATTTTCCT AGATCCACTT CTAGGAAAAA CTATTAGAGT ACTGGTTTTA GGGAATTGCA

                                              sau3AI
                                              mboI/ndeII[dam-]
                                              dpnI[dam+]  sau3AI
                                              dpnII[dam-] mboI/ndeII[dam-] thaI
                                             bstYI/xhoII  dpnI[dam+]       fnuDII/mvnI
                                       sau3AI  alwI[dam-] dpnII[dam-]      bstUI
                                       mboI/ndeII[dam-]   alwI[dam-]       bsh1236I
                  hgaI                 dpnI[dam+] mboII[dam-]             hinPI     fnu4HI
               ddeI                    dpnII[dam-]      bstYI/xhoII       hhaI/cfoI bbvI
6301 GAGTTTTCGT TCCACTGAGC GTCAGACCCC GTAGAAAAGA TCAAAGGATC TTCTTGAGAT CCTTTTTTTC TGCGCGTAAT CTGCTGCTTG CAAACAAAAA
     CTCAAAAGCA AGGTGACTCG CAGTCTGGGG CATCTTTTCT AGTTTCCTAG AAGAACTCTA GGAAAAAAAG ACGCGCATTA GACGACGAAC GTTTGTTTTT

                                   sau3AI
                                   mboI/ndeII[dam-]
                                   dpnI[dam+]
                                   dpnII[dam-]
                                  alwI[dam-]
                 aciI           mspI                                     bsrI             hinPI
          aciI  nspBII          hpaII      aluI                       maeIII    eco57I    hhaI/cfoI
6401 AACCACCGCT ACCAGCGGTG GTTTGTTTGC CGGATCAAGA GCTACCAACT CTTTTTCCGA AGGTAACTGG CTTCAGCAGA GCGCAGATAC CAAATACTGT
     TTGGTGGCGA TGGTCGCCAC CAAACAAACG GCCTAGTTCT CGATGGTTGA GAAAAAGGCT TCCATTGACC GAAGTCGTCT CGCGTCTATG GTTTATGACA

                                                                                            fnu4HI
                                                                                     alwNI  bbvI
                                                                                    bsrI  fnu4HI
         rmaI              haeIII/palI
         maeI       bslI  haeI            scfI     aciI        mnlI          maeIII      bbvI     bsrI
6501 CCTTCTAGTG TAGCCGTAGT TAGGCCACCA CTTCAAGAAC TCTGTAGCAC CGCCTACATA CCTCGCTCTG CTAATCCTGT TACCAGTGGC TGCTGCCAGT
     GGAAGATCAC ATCGGCATCA ATCCGGTGGT GAAGTTCTTG AGACATCGTG GCGGATGTAT GGAGCGAGAC GATTAGGACA ATGGTCACCG ACGACGGTCA
```

FIG. 3R

```
                        scrFI                                     aciI                         hgiAI/aspHI
                        nciI                                    nspBII                         bsp1286
                        mspI                       mspI        fnu4HI                          bsiHKAI
                        hpaII                      hpaII       bbvI                            bmyI
                        dsaV     pleI             bsaWI      hinPI  mcrI                       apaLI/snoI
                        cauII    hinfI         maeIII        hhaI/cfoI                         alw44I/snoI  aluI
6601 GGCGATAAGT CGTGTCTTAC CGGGTTGGAC TCAAGACGAT AGTTACCGGA TAAGGCGCAG CGGTCGGGCT GAACGGGGGG TTCGTGCACA CAGCCCAGCT
     CCGCTATTCA GCACAGAATG GCCCAACCTG AGTTCTGCTA TCAATGGCCT ATTCCGCGTC GCCAGCCCGA CTTGCCCCCC AAGCACGTGT GTCGGGTCGA

                                                                                                         mspI
                                                             hinPI                                       hpaII
                                                             hhaI/cfoI                                   bslI  fnu4HI
                             ddeI      scfI                haeII                           aciI        bsaWI  aciI
6701 TGGAGCGAAC GACCTACACC GAACTGAGAT ACCTACAGCG TGAGCATTGA GAAAGCGCCA CGCTTCCCGA AGGGAGAAAG GCGGACAGGT ATCCGGTAAG
     ACCTCGCTTG CTGGATGTGG CTTGACTCTA TGGATGTCGC ACTCGTAACT CTTTCGCGGT GCGAAGGGCT TCCCTCTTTC CGCCTGTCCA TAGGCCATTC

                                           scrFI
                                           mvaI          scrFI
                                           ecoRII  mvaI
                                           dsaV    ecoRII
                                           bstNI         dsaV
                           hinPI mnlI      bsaJI         bstNI                                                 taqI
                           hhaI/cfoI   aluI apyI[dcm+]   apyI[dcm+]                          mnlI drdI   hgaI
6801 CGGCAGGGTC GGAACAGGAG AGCGCACGAG GGAGCTTCCA GGGGGAAACG CCTGGTATCT TTATAGTCCT GTCGGGTTTC GCCACCTCTG ACTTGAGCGT
     GCCGTCCCAG CCTTGTCCTC TCGCGTGCTC CCTCGAAGGT CCCCCTTTGC GGACCATAGA AATATCAGGA CAGCCCAAAG CGGTGGAGAC TGAACTCGCA

                                                                                    haeIII/palI
                                                               haeIII/palI       scrFI
                                                              fnu4HI             mvaI bslI
                                                              aciI               ecoRII
                                                             thaI bslI           dsaV                   nlaIII
                                                             fnuDII/mvnI         bstNI                      nspI
                                    nlaIV                    bstUI               apyI[dcm+]   haeIII/palI  nspHI
                  sfaNI            aciI                      bsh1236I      nlaIV  haeI        haeI         aflIII
6901 CGATTTTTGT GATGCTCGTC AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT TCCTGGCCTT TTGCTGGCCT TTTGCTCACA
     GCTAAAAACA CTACGAGCAG TCCCCCCGCC TCGGATACCT TTTTGCGGTC GTTGCGCCGG AAAAATGCCA AGGACCGGAA AACGACCGGA AAACGAGTGT

                                                                                   fnu4HI             fnu4HI
                                                                                   bbvI               bbvI pleI
                             tfiI                                          bsrBI  aciI              hinPI  hinfI
                             hinfI                aciI         aluI        aciI  fnu4HI      mcrI   hhaI/cfoI
7001 TGTTCTTTCC TGCGTTATCC CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC TCGCCGCAGC CGAACGACCG AGCGCAGCGA
     ACAAGAAAGG ACGCAATAGG GGACTAAGAC ACCTATTGGC ATAATGGCGG AAACTCACTC GACTATGGCG AGCGGCGTCG GCTTGCTGGC TCGCGTCGCT
```

FIG. 3S

```
                                                          thaI
                                                          fnuDII/mvnI
                                                          bstUI
                                                          bsh1236I
                                                         hinPI
                                                         hhaI/cfoI
                                                        thaI
                                                        fnuDII/mvnI
                      sapI  hinPI                       bstUI   haeIII/palI      aluI
                      mboII hhaI/cfoI                   bsh1236I          tru9I  pvuII
                      earI/ksp632I              mnlI   bslI    eaeI  tfiI aseI/asnI/vspI
                 mnlI  aciI    haeII           aciI    aciI    cfrI  hinfI mseI  nspBII             bsrI
7101 GTCAGTGAGC GAGGAAGCGG AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATCCAGCT GGCACGACAG GTTTCCCGAC
     CAGTCACTCG CTCCTTCGCC TTCTCGCGGG TTATGCGTTT GGCGGAGAGG GGCGCGCAAC CGGCTAAGTA ATTAGGTCGA CCGTGCTGTC CAAAGGGCTG

                                                                scrFI
                                                                mvaI
                                                                ecoRII
                                                                dsaV
                                 tru9I                     nlaIV bstNI
                      hinPI      mseI       maeIII         hgiCI apyI[dcm+]             mspI
           aciI       hhaI/cfoI aseI/asnI/vspI  mnlI       banI bsaJI                   hpaII
7201 TGGAAAGCGG GCAGTGAGCG CAACGCAATT AATGTGAGTT ACCTCACTCA TTAGGCACCC CAGGCTTTAC ACTTTATGCT TCCGGCTCGT ATGTTGTGTG
     ACCTTTCGCC CGTCACTCGC GTTGCGTTAA TTACACTCAA TGGAGTGAGT AATCCGTGGG GTCCGAAATG TGAAATACGA AGGCCGAGCA TACAACACAC

                                                             tru9I
                                                             mseI
                                                            aseI/asnI/vspI
             aciI                                          xmnI
            bsrBI                    aluI      nlaIII      asp700
7301 GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCTA TGACCATGAT TACGAATTAA
     CTTAACACTC GCCTATTGTT AAAGTGTGTC CTTTGTCGAT ACTGGTACTA ATGCTTAATT
```

>length: 7360

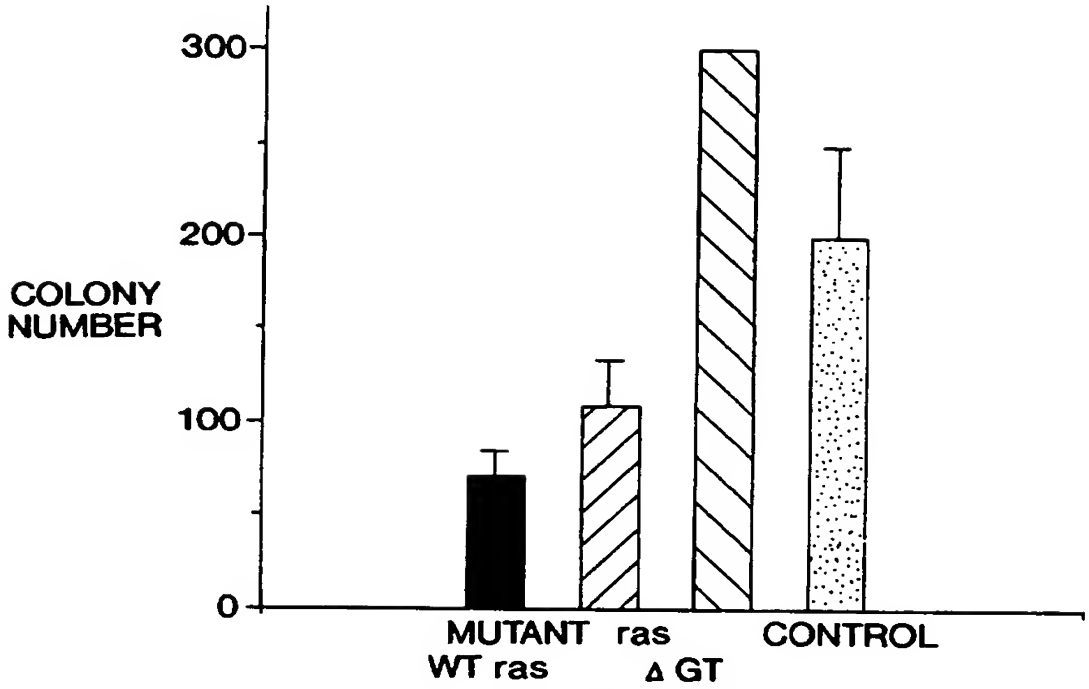


FIG. 4

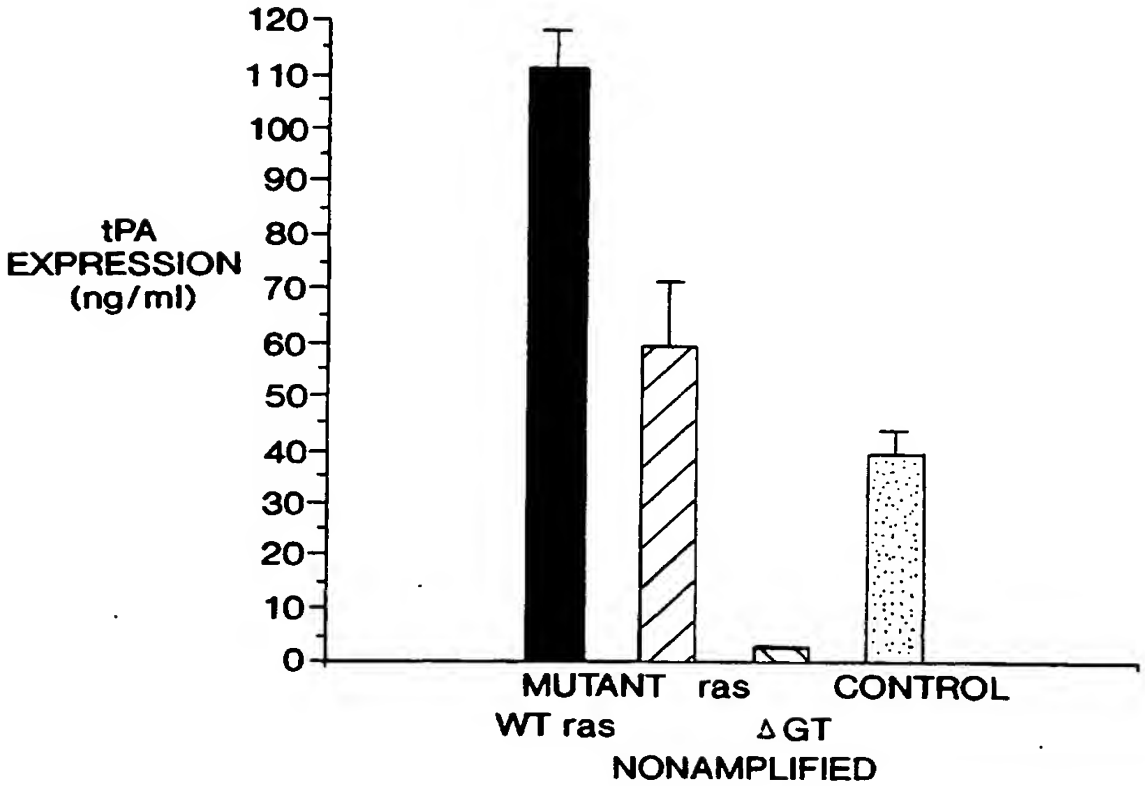


FIG. 5A

FIG. 10A

```
      aluI
     sstI
     sacI
     hgiJII
     hgiAI/aspHI
     ecl136II
     bsp1286
     bsiHKAI
     bmyI                       rmaI    tru9I
     banII                      maeI    mseI                                                         thaI
    taqI                       speI    aseI/asnI/vspI                             bslI              fnuDII/mvnJ
                                                                                                    bstUI
                                                                                                    bsh1236I
                                                                                                   aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                 scrFI
                 mvaI
                 ecoRII
                 dsaV
              aciI
            bglI bstNI                                     maeII
            sau96I                                       hinlI/acyI
            haeIII/palI     aciI                         ahaII/bsaHI
            asuI  apyI(dcm+)             aciI            aatII         maeII           maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                       maeII
     hinlI/acyI                                                                                  hinlI/acyI
     ahaII/bsaHI                                    rsaI                       rsaI              ahaII/bsaHI
     aatII                                 bglI     csp6I           ndeI       csp6I             aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

            scrFI
            mvaI
            ecoRII
        aciI                                                                                         nlaIII
      bglI  dsaV                                                                              styI
     sau96I bstNI          rsaI                                               maeII               ncoI
     haeIII/palI           csp6I                                   rsaI     snaBI                 dsaI hphI  aciI
     asuI   apyI(dcm+)   bsrI  nlaIII                              csp6I    bsaAI                 bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

FIG. 9R

```
                                                                   thaI
                                                                   fnuDII/mvnI
                                                                   bstUI
                                                                   bsh1236I
                                                                 hinPI
                                                                 hhaI/cfoI
                                                                thaI
                                                                fnuDII/mvnI
                         sapI  hinPI                            bstUI                 tru9I   aluI
                         mboII hhaI/cfoI                        bsh1236I haeIII/palI        pvuII
                         earI/ksp632I                   mnlI   bslI     eaeI  tfiI aseI/asnI/vspI
              mnlI   aciI     haeII                 aciI        aciI     cfrI  hinfI mseI   nspBII          bsrI
6301 AGTGAGCGAG GAAGCGGAAG AGCGCCCAAT ACGCAAACCG CCTCTCCCCG CGCGTTGGCC GATTCATTAA TCCAGCTGGC ACGACAGGTT TCCCGACTGG
     TCACTCGCTC CTTCGCCTTC TCGCGGGTTA TGCGTTTGGC GGAGAGGGGC GCGCAACCGG CTAAGTAATT AGGTCGACCG TGCTGTCCAA AGGGCTGACC

                                                                      scrFI
                                                                      mvaI
                                                                      ecoRII
                                                                      dsaV
                                   tru9I                         nlaIV bstNI
                     hinPI         mseI      maeIII              hgiCI apyI[dcm+]                mspI
          aciI       hhaI/cfoI    aseI/asnI/vspI mnlI            banI bsaJI                      hpaII
6401 AAAGCGGGCA GTGAGCGCAA CGCAATTAAT GTGAGTTACC TCACTCATTA GGCACCCCAG GCTTTACACT TTATGCTTCC GGCTCGTATG TTGTGTGGAA
     TTTCGCCCGT CACTCGCGTT GCGTTAATTA CACTCAATGG AGTGAGTAAT CCGTGGGGTC CGAAATGTGA AATACGAAGG CCGAGCATAC AACACACCTT

                                                                    tru9I
                                                                    mseI
                                                                   aseI/asnI/vspI
             aciI                                                  xmnI
           bsrBI                    aluI      nlaIII               asp700
6501 TTGTGAGCGG ATAACAATTT CACACAGGAA ACAGCTATGA CCATGATTAC GAATTAA
     AACACTCGCC TATTGTTAAA GTGTGTCCTT TGTCGATACT GGTACTAATG CTTAATT
```

>length: 6557

FIG. 9Q

```
                                                                                                      mspI
                                                             hinPI                                    hpaII
                                                             hhaI/cfoI                                bslI    fnu4HI
                           ddeI     scfI                    haeII                        aciI        bsaWI    aciI
5901 AGCGAACGAC CTACACCGAA CTGAGATACC TACAGCGTGA GCATTGAGAA AGCGCCACGC TTCCCGAAGG GAGAAAGGCG GACAGGTATC CGGTAAGCGG
     TCGCTTGCTG GATGTGGCTT GACTCTATGG ATGTCGCACT CGTAACTCTT TCGCGGTGCG AAGGGCTTCC CTCTTTCCGC CTGTCCATAG GCCATTCGCC

                                          scrFI
                                          mvaI          scrFI
                                          ecoRII  mvaI
                                          dsaV    ecoRII
                                          bstNI         dsaV
                        hinPI  mnlI       bsaJI         bstNI                                             taqI
                        hhaI/cfoI   aluI  apyI[dcm+]    apyI[dcm+]                            mnlI drdI    hgaI
6001 CAGGGTCGGA ACAGGAGAGC GCACGAGGGA GCTTCCAGGG GGAAACGCCT GGTATCTTTA TAGTCCTGTC GGGTTTCGCC ACCTCTGACT TGAGCGTCGA
     GTCCCAGCCT TGTCCTCTCG CGTGCTCCCT CGAAGGTCCC CCTTTGCGGA CCATAGAAAT ATCAGGACAG CCCAAAGCGG TGGAGACTGA ACTCGCAGCT

                                                                                   haeIII/palI
                                                               haeIII/palI     scrFI
                                                             fnu4HI            mvaI
                                                             aciI              ecoRII
                                                            thaI bslI          dsaV                    nlaIII
                                                            fnuDII/mvnI        bstNI bslI                   nspI
                                nlaIV                       bstUI              apyI[dcm+]      haeIII/palI  nspHI
            sfaNI             aciI                          bsh1236I       nlaIV  haeI        haeI          aflIII
6101 TTTTTGTGAT GCTCGTCAGG GGGGCGGAGC CTATGGAAAA ACGCCAGCAA CGCGGCCTTT TTACGGTTCC TGGCCTTTTG CTGGCCTTTT GCTCACATGT
     AAAAACACTA CGAGCAGTCC CCCCGCCTCG GATACCTTTT TGCGGTCGTT GCGCCGGAAA AATGCCAAGG ACCGGAAAAC GACCGGAAAA CGAGTGTACA

                                                                                    fnu4HI              fnu4HI
                                                                                    bbvI                bbvI pleI
                           tfiI                                           bsrBI   aciI               hinPI   hinfI
                           hinfI                   aciI          aluI     aciI  fnu4HI        mcrI   hhaI/cfoI
6201 TCTTTCCTGC GTTATCCCCT GATTCTGTGG ATAACCGTAT TACCGCCTTT GAGTGAGCTG ATACCGCTCG CCGCAGCCGA ACGACCGAGC GCAGCGAGTC
     AGAAAGGACG CAATAGGGGA CTAAGACACC TATTGGCATA ATGGCGGAAA CTCACTCGAC TATGGCGAGC GGCGTCGGCT TGCTGGCTCG CGTCGCTCAG
```

FIG. 9P

```
                                                      sau3AI
                                                      mboI/ndeII(dam-)
                                                      dpnI(dam+)  sau3AI              thaI
                                                      dpnII(dam-) mboI/ndeII(dam-)
                                                     bstYI/xhoII  dpnI(dam+)          fnuDII/mvnI
                                              sau3AI alwI(dam-)   dpnII(dam-)         bstUI
                                              mboI/ndeII(dam-)    alwI(dam-)          bsh1236I
                           hgaI               dpnI(dam+) mboII(dam-)                 hinPI       fnu4HI
                       ddeI                   dpnII(dam-)        bstYI/xhoII         hhaI/cfoI   bbvI
5501 TTTTCGTTCC ACTGAGCGTC AGACCCCGTA GAAAAGATCA AAGGATCTTC TTGAGATCCT TTTTTTCTGC GCGTAATCTG CTGCTTGCAA ACAAAAAAAC
     AAAAGCAAGG TGACTCGCAG TCTGGGGCAT CTTTTCTAGT TTCCTAGAAG AACTCTAGGA AAAAAAGACG CGCATTAGAC GACGAACGTT TGTTTTTTTG

                                        sau3AI
                                        mboI/ndeII(dam-)
                                        dpnI(dam+)
                                        dpnII(dam-)
                                       alwI(dam-)
                       aciI            mspI                                         bsrI           hinPI
         aciI     nspBII               hpaII        aluI                         maeIII   eco57I   hhaI/cfoI
5601 CACCGCTACC AGCGGTGGTT TGTTTGCCGG ATCAAGAGCT ACCAACTCTT TTTCCGAAGG TAACTGGCTT CAGCAGAGCG CAGATACCAA ATACTGTCCT
     GTGGCGATGG TCGCCACCAA ACAAACGGCC TAGTTCTCGA TGGTTGAGAA AAAGGCTTCC ATTGACCGAA GTCGTCTCGC GTCTATGGTT TATGACAGGA

                                                                                                         fnu4HI
                                                                                                 alwNI   bbvI
       rmaI               haeIII/palI                                                          bsrI   fnu4HI
       maeI        bslI  haeI                  scfI     aciI         mnlI                   maeIII    bbvI     bsrI
5701 TCTAGTGTAG CCGTAGTTAG GCCACCACTT CAAGAACTCT GTAGCACCGC CTACATACCT CGCTCTGCTA ATCCTGTTAC CAGTGGCTGC TGCCAGTGGC
     AGATCACATC GGCATCAATC CGGTGGTGAA GTTCTTGAGA CATCGTGGCG GATGTATGGA GCGAGACGAT TAGGACAATG GTCACCGACG ACGGTCACCG

                       scrFI                                             aciI                    hgiAI/aspHI
                       nciI                                            nspBII                    bsp1286
                       mspI                                 mspI      fnu4HI                     bsiHKAI
                       hpaII                                hpaII     bbvI                       bmyI
                       dsaV    pleI                        bsaWI    hinPI mcrI                   apaLI/snoI
                       cauII   hinfI                   maeIII       hhaI/cfoI                    alw44I/snoI  aluI
5801 GATAAGTCGT GTCTTACCGG GTTGGACTCA AGACGATAGT TACCGGATAA GGCGCAGCGG TCGGGCTGAA CGGGGGGTTC GTGCACACAG CCCAGCTTGG
     CTATTCAGCA CAGAATGGCC CAACCTGAGT TCTGCTATCA ATGGCCTATT CCGCGTCGCC AGCCCGACTT GCCCCCCAAG CACGTGTGTC GGGTCGAACC
```

FIG. 90

```
                                                                                           hinPI
         mspI                                                                             mstI
          hpaII                                                                            aviII/fspI    bsrI
         bsaWI                                                          fnu4HI         maeII hhaI/cfoI  tru9I
      nlaIV    aluI                                 maeIII     sfaNI    bbvI          psp1406I          mseI
5001 ATCGTTGGGA ACCGGAGCTG AATGAAGCCA TACCAAACGA CGAGCGTGAC ACCACGATGC CAGCAGCAAT GGCAACAACG TTGCGCAAAC TATTAACTGG
     TAGCAACCCT TGGCCTCGAC TTACTTCGGT ATGGTTTGCT GCTCGCACTG TGGTGCTACG GTCGTCGTTA CCGTTGTTGC AACGCGTTTG ATAATTGACC

                  mspI
                  hpaII                                                                     bglI
                 scrFI                                                                      sau96I
           aluI  nciI      tru9I    fokI                           sau96I                   haeIII/palI
          rmaI   dsaV      mseI   bsrI        aciI                 avaII          hinPI     asuI     mspI
          maeI   cauII     aseI/asnI/vspI  mnlI                    asuI           hhaI/cfoI          hpaII
5101 CGAACTACTT ACTCTAGCTT CCCGGCAACA ATTAATAGAC TGGATGGAGG CGGATAAAGT TGCAGGACCA CTTCTGCGCT CGGCCCTTCC GGCTGGCTGG
     GCTTGATGAA TGAGATCGAA GGGCCGTTGT TAATTATCTG ACCTACCTCC GCCTATTTCA ACGTCCTGGT GAAGACGCGA GCCGGGAAGG CCGACCGACC

                   mspI             thaI
                   hpaII            fnuDII/mvnI                 haeIII/palI
                  cfr10I            bstUI                      sau96I
              nlaIV  hphI         bsmAI aciI          fnu4HI  nlaIV
             gsuI/bpmI            bsaI bsh1236I       bbvI bsrI asuI              mnlI
5201 TTTATTGCTG ATAAATCTGG AGCCGGTGAG CGTGGGTCTC GCGGTATCAT TGCAGCACTG GGGCCAGATG GTAAGCCCTC CCGTATCGTA GTTATCTACA
     AAATAACGAC TATTTAGACC TCGGCCACTC GCACCCAGAG CGCCATAGTA ACGTCGTGAC CCCGGTCTAC CATTCGGGAG GGCATAGCAT CAATAGATGT

                                               ddeI
                                            sau3AI       nlaIV
                                            mboI/ndeII[dam-]  mnlI
        pleI                                dpnI[dam+]   hgiCI          tru9I
        hinfI                               dpnII[dam-]  banI           mseI       maeIII
     eam1105I            fokI
5301 CGACGGGGAG TCAGGCAACT ATGGATGAAC GAAATAGACA GATCGCTGAG ATAGGTGCCT CACTGATTAA GCATTGGTAA CTGTCAGACC AAGTTTACTC
     GCTGCCCCTC AGTCCGTTGA TACCTACTTG CTTTATCTGT CTAGCGACTC TATCCACGGA GTGACTAATT CGTAACCATT GACAGTCTGG TTCAAATGAG

                                                  rmaI       sau3AI
                                                sau3AI hphI  mboI/ndeII[dam-]
                                                mboI/ndeII[dam-]
                                                dpnI[dam+]   dpnI[dam+]
                                          tru9I dpnII[dam-]  dpnII[dam-]
                 tru9I                   ahaIII/draI maeI    alwI[dam-]            nlaIII                maeII
                 mseI             tru9I       bstYI/xhoII  bstYI/xhoII             rcaI               tru9I
                ahaIII/draI       mseI mseI   alwI[dam-] mboII[dam-]               bspHI              mseI
5401 ATATATACTT TAGATTGATT TAAAACTTCA TTTTTAATTT AAAAGGATCT AGGTGAAGAT CCTTTTTGAT AATCTCATGA CCAAAATCCC TTAACGTGAG
     TATATATGAA ATCTAACTAA ATTTTGAAGT AAAAATTAAA TTTTCCTAGA TCCACTTCTA GGAAAAACTA TTAGAGTACT GGTTTTAGGG AATTGCACTC
```

FIG. 9N

```
                                          hgiAI/aspHI
                                          bsp1286
                               sau3AI     bsiHKAI                      sau3AI  nspBII    sau3AI
                               mboI/ndeII(dam-)                        mboI/ndeII(dam-)  mboI/ndeII(dam-)
                               dpnI(dam+) bmyI                         dpnI(dam+)        dpnI(dam+)
                               dpnII(dam-)                           bstYI/xhoII         dpnII(dam-)
                         eco57I           apaLI/snoI              bsrI dpnII(dam-)       alwI(dam-)
       hphI          sfaNI  mboII(dam-) alw44I/snoI  maeIII taqI alwI(dam-)   aciI  bstYI/xhoII                  mboII
4601 CTGGTGAAAG TAAAAGATGC TGAAGATCAG TTGGGTGCAC GAGTGGGTTA CATCGAACTG GATCTCAACA GCGGTAAGAT CCTTGAGAGT TTTCGCCCCG
     GACCACTTTC ATTTTCTACG ACTTCTAGTC AACCCACGTG CTCACCCAAT GTAGCTTGAC CTAGAGTTGT CGCCATTCTA GGAACTCTCA AAAGCGGGGC

                                                                                scrFI
                                                                                nciI
                                                        aciI                    mspI
                                                       thaI                     hpaII
                                                       fnuDII/mvnI              dsaV
         maeII               hgiAI/aspHI               bstUI                    cauII
        psp1406I             bsp1286   tru9I           bsh1236I             hinlI/acyI
       xmnI                  bsiHKAI   mseI           hinPI                 hgaI                        aciI
       asp700                bmyI     ahaIII/draI     hhaI/cfoI             ahaII/bsaHI    bcgI  mcrI  fnu4HI
4701 AAGAACGTTT TCCAATGATG AGCACTTTTA AAGTTCTGCT ATGTGGCGCG GTATTATCCC GTGATGACGC CGGGCAAGAG CAACTCGGTC GCCGCATACA
     TTCTTGCAAA AGGTTACTAC TCGTGAAAAT TTCAAGACGA TACACCGCGC CATAATAGGG CACTACTGCG GCCCGTTCTC GTTGAGCCAG CGGCGTATGT

                               rsaI
                               csp6I   bsrI                                                 fnu4HI
          ddeI                scaI hphI   maeIII      sfaNI     fokI   nlaIII               bbvI        nlaIII
4801 CTATTCTCAG AATGACTTGG TTGAGTACTC ACCAGTCACA GAAAAGCATC TTACGGATGG CATGACAGTA AGAGAATTAT GCAGTGCTGC CATAACCATG
     GATAAGAGTC TTACTGAACC AACTCATGAG TGGTCAGTGT CTTTTCGTAG AATGCCTACC GTACTGTCAT TCTCTTAATA CGTCACGACG GTATTGGTAC

                                                   sau96I
                                                   avaII                                              maeIII
                                           sau3AI  asuI                                            nlaIII
                    haeIII/palI            mboI/ndeII(dam-)                                     sau3AI
                   eaeI                    dpnI(dam+)                                           mboI/ndeII(dam-)  sau3AI
                   cfrI                    dpnII(dam-)                                          dpnI(dam+)        mboI/ndeII(dam-)
                  fnu4HI                  pvuI/bspCI                                            dpnII(dam-)       dpnI(dam+)
                  aciI                    mcrI   mnlI         aluI  aciI                 nlaIII alwI(dam-)        dpnII(dam-)
4901 AGTGATAACA CTGCGGCCAA CTTACTTCTG ACAACGATCG GAGGACCGAA GGAGCTAACC GCTTTTTTGC ACAACATGGG GGATCATGTA ACTCGCCTTG
     TCACTATTGT GACGCCGGTT GAATGAAGAC TGTTGCTAGC CTCCTGGCTT CCTCGATTGG CGAAAAAACG TGTTGTACCC CCTAGTACAT TGAGCGGAAC
```

FIG. 9M

```
                                                                                                           hinPI
                                                                                                          hhaI/cfoI
                                                                                                          thaI
                                                                       ·hinPI                             fnuDII/mvnI
          aciI                                       maeIII           fnu4HI                              bstUI
         fnu4HI    tru9I                           maeII bsrI  nlaIII  hhaI/cfoI                nspBII    bsh1236I
      sfaNI        mseI         aciI               bsaAI tth111I/aspI bbvI             aciI     aciI  hgaI
4101 ACAATCTGCT CTGATGCCGC ATAGTTAAGC CAACTCCGCT ATCGCTACGT GACTGGGTCA TGGCTGCGCC CCGACACCCG CCAACACCCG CTGACGCGCC
     TGTTAGACGA GACTACGGCG TATCAATTCG GTTGAGGCGA TAGCGATGCA CTGACCCAGT ACCGACGCGG GGCTGTGGGC GGTTGTGGGC GACTGCGCGG

                                                                                                               thaI
                                                                                                               fnuDII/mvnI
                                                                                                               bstUI
                                                              scrFI                                            bsh1236I
                mspI                                          nciI                                            hinPI
                hpaII                                         mspI                                            hhaI/cfoI
               scrFI                                     bsmAI          nspI                                 thaI
               nciI    aciI                             esp3I hpaII fnu4HI                                   fnuDII/mvnI
               dsaV sfaNI                              bslI  dsaV aluI nspHI                                 bstUI
   drdI        cauII fokI                  aluI maeIII      cauII bbvI nlaIII   mnlI    hphI       hphI     bsh1236I
4201 CTGACGGGCT TGTCTGCTCC CGGCATCCGC TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT CACCGTCATC ACCGAAACGC
     GACTGCCCGA ACAGACGAGG GCCGTAGGCG AATGTCTGTT CGACACTGGC AGAGGCCCTC GACGTACACA GTCTCCAAAA GTGGCAGTAG TGGCTTTGCG

                                mnlI
                              haeIII/palI                                                  maeII
               mboII         sau96I                               nlaIII                  hinlI/acyI
               bpuAI         asuI                           tru9I rcaI                    ahaII/bsaHI
   mnlI        bbsI         eco0109I/draII                  mseI  bspHI              ddeI aatII
4301 GCGAGGCAGT ATTCTTGAAG ACGAAAGGGC CTCGTGATAC GCCTATTTTT ATAGGTTAAT GTCATGATAA TAATGGTTTC TTAGACGTCA GGTGGCACTT
     CGCTCCGTCA TAAGAACTTC TGCTTTCCCG GAGCACTATG CGGATAAAAA TATCCAATTA CAGTACTATT ATTACCAAAG AATCTGCAGT CCACCGTGAA

                nlaIV
              aciI
             thaI
             fnuDII/mvnI
             bstUI                                                   rcaI
             bsh1236I                                                bspHI
            hinPI                                                 bsrBI   bsmAI
            hhaI/cfoI                                             aciI nlaIII                          sspI
4401 TTCGGGGAAA TGTGCGCGGA ACCCCTATTT GTTTATTTTT CTAAATACAT TCAAATATGT ATCCGCTCAT GAGACAATAA CCCTGATAAA TGCTTCAATA
     AAGCCCCTTT ACACGCGCCT TGGGGATAAA CAAATAAAAA GATTTATGTA AGTTTATACA TAGGCGAGTA CTCTGTTATT GGGACTATTT ACGAAGTTAT

            mboII                                                 fnu4HI
            earI/ksp632I                                          aciI                     hphI
4501 ATATTGAAAA AGGAAGAGTA TGAGTATTCA ACATTTCCGT GTCGCCCTTA TTCCCTTTTT TGCGGCATTT TGCCTTCCTG TTTTTGCTCA CCCAGAAACG
     TATAACTTTT TCCTTCTCAT ACTCATAAGT TGTAAAGGCA CAGCGGGAAT AAGGGAAAAA ACGCCGTAAA ACGGAAGGAC AAAAACGAGT GGGTCTTTGC
```

FIG. 9L

```
                                                                           aciI
                                   hinPI                                 fnu4HI                 fnu4HI
                                   hhaI/cfoI                             aciI                 hinPI
                                  thaI                                  thaI                 hhaI/cfoI
                                  fnuDII/mvnI                           fnuDII/mvnI          thaI
                                  bstUI           hinPI                 bstUI                fnuDII/mvnI
                                  bsh1236I        hhaI/cfoI hinPI       bsh1236I             bstUI                         hinPI
                               rsaI       scfI fnu4HI    tru9I bsh1236I                      bsh1236I    aciI              hhaI/cfoI
      aciI  maeII              csp6I    bslI   aciI      mseI hhaI/cfoI          maeIII  bbvI  maeIII                     haeII
3601 CACCGCATAC GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG
     GTGGCGTATG CAGTTTCGTT GGTATCATGC GCGGGACATC GCCGCGTAAT TCGCGCCGCC CACACCACCA ATGCGCGTCG CACTGGCGAT GTGAACGGTC

           hinPI                                                                                nlaIV
           hhaI/cfoI                                           mspI                            hgiJII
          haeII                                                hpaII                           bsp1286
        rmaI    bsrBI                                         naeI                             bmyI
        maeI    aciI            mboII                 maeII  cfr10I             aluI           banII         nlaIV
3701 CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC TTTAGGGTTC
     GCGGGATCGC GGGCGAGGAA AGCGAAAGAA GGGAAGGAAA GAGCGGTGCA AGCGGCCGAA AGGGGCAGTT CGAGATTTAG CCCCCGAGGG AAATCCCAAG

                 nlaIV                                     maeII    haeIII/palI
                 hgiCI  taqI                              draIII   sau96I                                  maeII
                 banI mnlI                        hphI    bsaAI    asuI                                   drdI
3801 CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA
     GCTAAATCAC GAAATGCCGT GGAGCTGGGG TTTTTTGAAC TAAACCCACT ACCAAGTGCA TCACCCGGTA GCGGGACTAT CTGCCAAAAA GCGGGAAACT

         pleI          tru9I     pleI                                    bslI
         hinfI  maeII  mseI      hinfI        bsrI                      bslI    avaI
3901 CGTTGGAGTC CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG CTATTCTTTT GATTTATAAG GGATTTTGCC
     GCAACCTCAG GTGCAAGAAA TTATCACCTG AGAACAAGGT TTGACCTTGT TGTGAGTTGG GATAGAGCCC GATAAGAAAA CTAAATATTC CCTAAAACGG

                                                          thaI                                       hgiAI/aspHI
                                                          fnuDII/mvnI                                bsp1286
                                                          bstUI                    maeII             bsiHKAI
                 tru9I                                 tru9I                    tru9I                bmyI    ddeI
                 mseI               tru9I              mseI    apoI tru9I       mseI                 apaLI/snoI  rsaI
        haeIII/palI           aluI  mseI      apoI    bsh1236I mseI     sspI  psp1406I               alw44I/snoI csp6I
4001 GATTTCGGCC TATTGGTTAA AAAATGAGCT GATTTAACAA AAATTTAACG CGAATTTTAA CAAAATATTA ACGTTTACAA TTTTATGGTG CACTCTCAGT
     CTAAAGCCGG ATAACCAATT TTTTACTCGA CTAAATTGTT TTTAAATTGC GCTTAAAATT GTTTTATAAT TGCAAATGTT AAAATACCAC GTGAGAGTCA
```

FIG. 9K

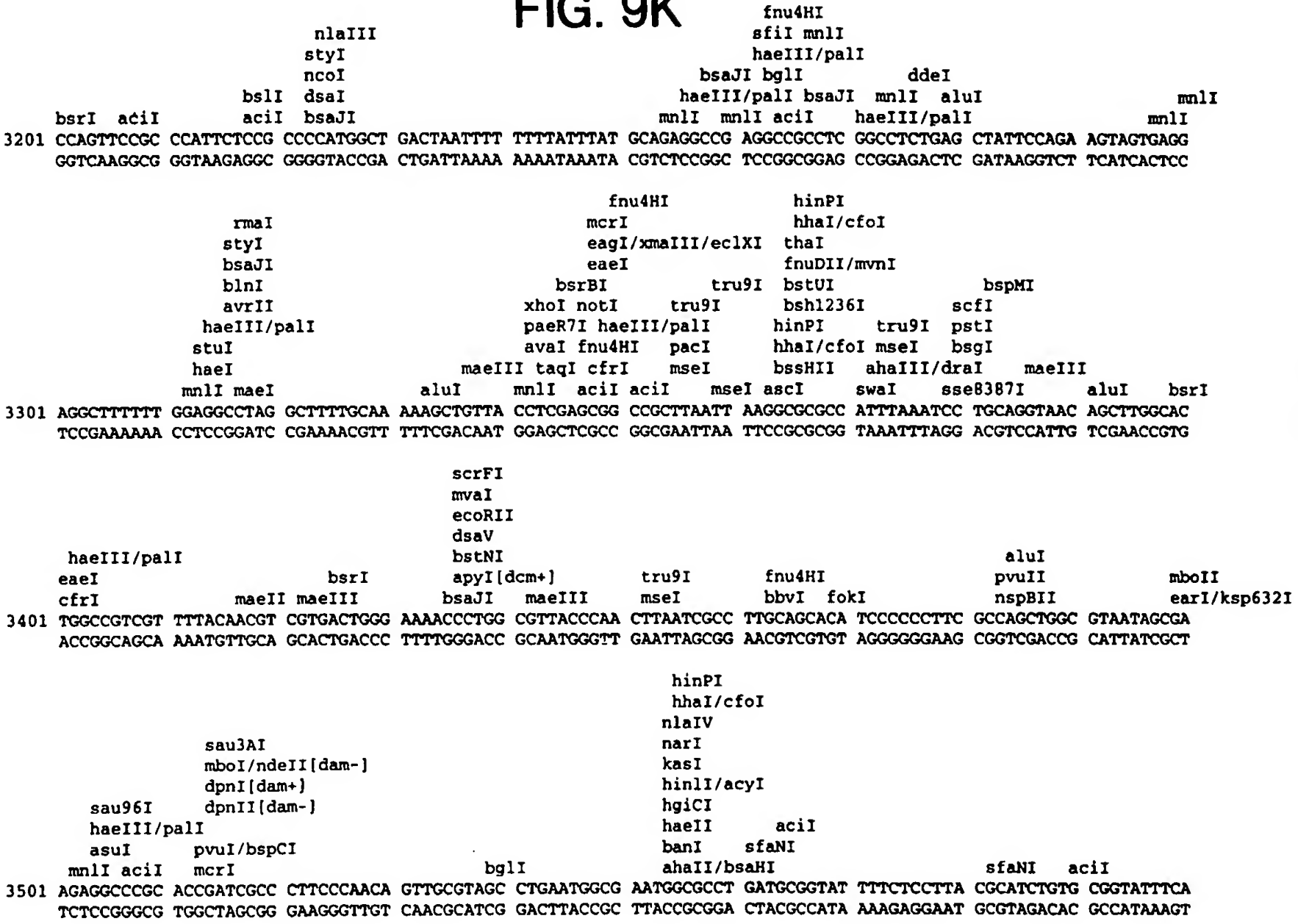


FIG. 9J

```
                                                                                        taqI(dam-)
                                                                                         claI/bsp106(dam-)
                                                                                        sau3AI
                                                                                        mboI/ndeII(dam-)
                                                                                        dpnI(dam+)
                                            rmaI                                        dpnII(dam-)
         sfaNI    apoI                 bsmI maeI                               nlaIII  alwI(dam-)
2801 AAAGCAATAG CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT
     TTTCGTTATC GTAGTGTTTA AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA

          tru9I
       xmnI                                                         rsaI
  sau3AI  aseI/asnI/vspI    styI                                    csp6I
  mboI/ndeII(dam-)          ncoI                                   nlaIV
  dpnI(dam+)           fnu4HI   haeI                               kpnI
  dpnII(dam-)          bbvI  dsaI haeIII/palI                      hgiCI
 pvuI/bspCI mseI     hinPI  bsaJI                                  banI                alwI
 mcrI   asp700       hhaI/cfoI nlaIII     mnlI      mnlI           asp718    mnlI      pvuII
                                                                   acc65I  ddeI aciI   nspBII
2901 CGATCGGGAA TTAATTCGGC GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA
     GCTAGCCCTT AATTAAGCCG CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT

                                nlaIV
                             scrFI                                          scrFI
                             mvaI                         ppu10I               mvaI
                             ecoRII                   nsiI/avaIII           ecoRII
                             dsaV                    nlaIII                 dsaV
                             bstNI                      sphI                   bstNI
                             apyI(dcm+)                 nspI sfaNI             apyI(dcm+)
                            bsaJI                       nspHI                 sexAI          bsaJI
3001 TGTGTGTCAG TTAGGGTGTG GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC
     ACACACAGTC AATCCCACAC CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG

       nlaIV                          sfaNI
     scrFI                        ppu10I
     mvaI                              nsiI/avaIII
     ecoRII                      nlaIII
     dsaV                       sphI
     bstNI                           nspI                                               aciI
     apyI(dcm+)                      nspHI                  aciI        aciI  fokI              aciI
3101 CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC
     GGTCCGAGGG GTCGTCCGTC TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG
```

FIG. 9I

```
                                           scrFI
                                           nciI
                                           mspI
                                           hpaII
                                           dsaV
                                           cauII
                                          xmaI/pspAI
                                          smaI
                                          scrFI                    scrFI
                                          nciI                    mvaI                scrFI
                                          dsaV                    ecoRII        mvaI
                                          cauII                   dsaV          ecoRII
                       rsaI            fokI    mboII              bstNI               dsaV
                       csp6I        bslI  bsaJI                   apyI[dcm+]          bstNI
      avaI            bsp1407I     bslI   avaI earI/ksp632I      sexAI          bspMI apyI[dcm+]
2401 CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG AAGAGATGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG
     GGGGCTCTTG GTGTCCACAT GTGGGACGGG GGTAGGGCCC TTCTCTACTG GTTCTTGGTC CAGTCGGACT GGACGGACCA GTTTCCGAAG ATAGGGTCGC

           dsaI                         mspI
           bslI                    fnu4HI                                      pleI                  mnlI
           bsaJI                   bbvI hpaII                     mnlI         hinfI     nlaIV   mboII   scfI    aluI
2501 ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA
     TGTAGCGGCA CCTCACCCTC TCGTTACCCG TCGGCCTCTT GTTGATGTTC TGGTGCGGAG GGCACGACCT GAGGCTGCCG AGGAAGAAGG AGATGTCGTT

                                             bpuAI
                                             bbsI                   nlaIII
          dsaI                             maeII                 ppu10I                             sapI
          bsaJI                fnu4HI    xmnI mboII              nsiI/avaIII                        mboII  mnlI
       hphI            bspMI   bbvI      asp700      nlaIII     sfaNI   mnlI                        earI/ksp632I bslI
2601 GCTCACCGTG GACAAGAGCA GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA GAGCCTCTCC
     CGAGTGGCAC CTGTTCTCGT CCACCGTCGT CCCCTTGCAG AAGAGTACGA GGCACTACGT ACTCCGAGAC GTGTTGGTGA TGTGCGTCTT CTCGGAGAGG

                                                                   styI
           scrFI                           taqI                 aciI
           nciI                           salI                 fnu4HI   sau96I
           mspI                 rmaI  hincII/hindII       sfiI ncoI haeIII/palI
           hpaII                maeI  accI   scfI         haeIII/palI
           dsaV             sau96I  pleI     pstI        eaeI  dsaI asuI                 aluI
           cauII            haeIII/palI      bsgI   aluI cfrI  bsaJI                  fnu4HI
       bsmAI                    asuI    hinfI bspMI   hindIII bglI nlaIII                 bbvI           maeIII
2701 CTGTCTCCGG GTAAATGAGT GCGACGGCCC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT
     GACAGAGGCC CATTTACTCA CGCTGCCGGG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA
```

FIG. 9H

```
                                             sau96I
                                             nlaIV
                                             avaII
                                           mspI
                                          scrFI
                                          nciI
                                    sau3AI hpaII
                                    mboI/ndeII[dam-]
                                    dpnI[dam+]                 nlaIII
                                 nlaIII   dsaV         mnlI   nspI                             drdI  mnlI
                                rcaI      cauII      ddeI maeIII                           mboII   ddeI
                   styI         bspHI[dam-]   asuI  eco81I    nspHI                        bpuAI  eco81I
                   bsaJI      mnlI  dpnII[dam-]     bsu36I/mstII/sauI         maeII       bbsI   bsu36I/mstII/sauI
2101 TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA
     AAGGGGGGTT TTGGGTTCCT GTGGGAGTAC TAGAGGGCCT GGGGACTCCA GTGTACGCAC CACCACCTGC ACTCGGTGCT TCTGGGACTC CAGTTCAAGT

                                                        aciI
                                                       thaI
                                                       fnuDII/mvnI
                                                       bstUI
                                                       bsh1236I
                                                      sacII/sstII
                                                      nspBII
                                                      kspI
           maeII                                      dsaI                         rsaI
         rsaI                                         bsaJI                        csp6I
         csp6I                                        aciI           rsaI       maeII                 mnlI      ecoNI
     bsrI bsaAI            mnlI                      fnu4HI  mnlI    csp6I     bsaAI              hgaI   hphI   bslI
2201 ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT
     TGACCATGCA CCTGCCGCAC CTCCACGTAT TACGGTTCTG TTTCGGCGCC CTCCTCGTCA TGTTGTCGTG CATGGCACAC CAGTCGCAGG AGTGGCAGGA

        scrFI
        mvaI bsrI
        ecoRII
        dsaV
        bstNI                rsaI        bsmAI                                                        fnu4HI
        apyI[dcm+]      csp6I        bsaI             mnlI           taqI                           bbvI
2301 GCACCAGGAC TGGCTGAATG GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA TCTCCAAAGC CAAAGGGCAG
     CGTGGTCCTG ACCGACTTAC CGTTCCTCAT GTTCACGTTC CAGAGGTTGT TTCGGGAGGG TCGGGGGTAG CTCTTTTGGT AGAGGTTTCG GTTTCCCGTC
```

FIG. 9G

```
                                                   hinPI
                                                        nlaIV
         scrFI                                    narI
         mvaI                                           kasI              hgiAI/aspHI
         ecoRII                                   hinlI/acyI        bsp1286     mspI
         ecoNI                 hphI                     hgiCI             bsiHKAI      hpaII
         dsaV                      mspI                 haeII             bmyI        scrFI
         bstNI                     hpaII                banI        fnu4HI            nciI
         bslI                    cfr10I                 ahaII/bsaHI aciI  apaLI/snoI  dsaV
         apyI[dcm+]              bsaWI  tth111I/aspI    hhaI/cfoI nspBII  alw44I/snoI cauII
     fnu4HI                bslI  ageI maeIII       ddeI                                          scfI
     bbvI
1801 GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT
     CCGACGGACC AGTTCCTGAT GAAGGGGCTT GGCCACTGCC ACAGCACCTT GAGTCCGCGG GACTGGTCGC CGCACGTGTG GAAGGGCCGA CAGGATGTCA

                                                           nlaIV
     ddeI pleI                                      fnu4HI hgiCI
     mnlI  hinfI                              mnlI  bbvI   banI                       tfiI
     eco81I         mnlI  fnu4HI  maeIII bsp1286 rmaI     bsp1286                     hinfI              styI
     bsu36I/mstII/sauI ddeI bbvI   hphI   bmyI    maeI aluI bmyI                  maeII                  bsaJI
1901 CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACTGTGCC CTCTAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC
     GGAGTCCTGA GATGAGGGAG TCGTCGCACC ACTGACACGG GAGATCGTCG AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG

                                                                                        eam1105I
                                                                                       sau96I
                                                                                 scrFI
                                                                                 mvaI    avaII
                                                                                 ecoRII
                    hgiJII                                                       dsaV
                    bsp1286                    nlaIII                            bstNI   asuI      mboII mboII
                    bmyI                       nspI        bsp1286               bsaJI  nlaIV     bpuAI earI/ksp632I
                    banII          maeIII      nspHI       bmyI  alwNI           apyI[dcm+]       bbsI mnlI
2001 CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG GGGGACCGTC AGTCTTCCTC
     GTTCCACCTG TTCTTTCAAC TCGGGTTTAG AACACTGTTT TGAGTGTGTA CGGGTGGCAC GGGTCGTGGA CTTGAGGACC CCCCTGGCAG TCAGAAGGAG
```

FIG. 9F

```
                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                                                                          dsaV
                                                                          bstNI
                                                                          apyI[dcm+]
                                                                        hinPI
                                                                        hhaI/cfoI
                                                    mnlI               nlaIV
                                                  xhoI                 narI
                                                  paeR7I               kasI
                                                  avaI                 hinlI/acyI
        scfI                                   hgiAI/aspHI             hgiCI
        pstI                                   bsp1286                 haeII
        bsgI                 mnlI              bsiHKAI   fnu4HI        banI
      bspMI                ddeI drdI           bmyI taqI bbvI          ahaII/bsaHI
1601 ACCTGCAGAT GAACAGCCTG CGTGCTGAGG ACACTGCCGT CTATTATTGT GCTCGAGGCA GCCACTATTT CGGCGCCTGG CACTTCGCCG TGTGGGGTCA
     TGGACGTCTA CTTGTCGGAC GCACGACTCC TGTGACGGCA GATAATAACA CGAGCTCCGT CGGTGATAAA GCCGCGGACC GTGAAGCGGC ACACCCCAGT

                                      sau96I
                                      haeIII/palI
                                     sau96I
                                     nlaIV                                                                 scrFI
                                     hgiJII                nlaIV                                           mvaI
                                     bsp1286               hgiCI                                           ecoRII
                                                                                                           dsaV
          scrFI                   bsp120I              banI                                           bstNI
          mvaI                        bmyI               scrFI                                         bsaJI
          ecoRII                  banII             mvaI                                            sau96I
          dsaV                    asuI              ecoRII                                      haeIII/palI
          bstNI hphI                  apaI        mboII   dsaV        hgiAI/aspHI                   asuI
          apyI[dcm+] bsmAI    haeIII/palI eco0109I/draII  bstNI           bsp1286                      fnu4HI
         bsaJI maeIII     mnlI      styI asuI     bpuAI   apyI[dcm+]  mnlI   bsiHKAI       bsp1286 aciI apyI[dcm+]
   nlaIV   bstEII esp3I bsaJI mnlI  bsaJI         bbsI   bsaJI     mnlI      bmyI mnlI     bmyI nspBII bsaJI
1701 AGGAACCCTG GTCACCGTCT CCTCGGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC AGCGGCCCTG
     TCCTTGGGAC CAGTGGCAGA GGAGCCGGAG GTGGTTCCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG TTCTCGTGGA GACCCCCGTG TCGCCGGGAC
```

FIG. 9E

```
                                                                                                  scrFI
                                                                                                  nciI
                                                                                                  mspI
                                                                                                  hpaII
                                                                                                  dsaV
                                                                                                  cauII
                                                                                                  bslI
                                                                                                 xmaI/pspAI
                                                                                                 smaI
                                                                                                 scrFI
                                                                                                 nciI
                                                                                                 dsaV
           hgiJII                                                                                cauII        scrFI
           bsp1286                                                                            sau96I          mvaI
           bmyI                                                                     sau3AI    nlaIV           ecoRII
                                                               mspI                 mboI/ndeII[dam-]          dsaV
      scrFI                                               hpaII                dpnI[dam+]  bslI         bstNI
      mvaI banII                                               mroI                 dpnII[dam-] bsaJI         bslI
      ecoRII                                              bspMII               nlaIV      haeIII/palI   apyI[dcm+]
      dsaV                                                bspEI                bstYI/xhoII  avaI     sau96I
      bstNI                                                     bsaWI                 bamHI     eco0109I/draII haeIII/palI
      bsaJI                                                   mnlI                    alwI[dam-] asuI          asuI
      apyI[dcm+]                    alwNI                hphI  accIII    aluI   bsrI alwI[dam-]  bsaJI     eco0109I/draII
1401 GCCAGGGGGC TCACTCCGTT TGTCCTGTGC AGTTTCTGGC TACTCCATCA CCTCCGGATA TAGCTGGAAC TGGATCCGTC AGGCCCCGGG TAAGGGCCTG
     CGGTCCCCCG AGTGAGGCAA ACAGGACACG TCAAAGACCG ATGAGGTAGT GGAGGCCTAT ATCGACCTTG ACCTAGGCAG TCCGGGGCCC ATTCCCGGAC

                                   sau3AI
                                   mboI/ndeII[dam-]
                                   dpnI[dam+]                                                  thaI
                        maeII      dpnII[dam-]                                                 fnuDII/mvnI
                  taqI snaBI      alwI[dam-]                hgaI     haeIII/palI               bstUI  tfiI
                 claI/bsp106    mspI                     rmaI       sau96I                     bsh1236I
              sfaNI    bsaAI    hpaII  taqI[dam-]        maeI       asuI                      nruI    hinfI
1501 GAATGGGTTG CATCGATTAC GTATGCCGGA TCGACTAACT ATAACCCTAG CGTCAAGGGC CGTATCACTA TAAGTCGCGA CGATTCCAAA AACACATTCT
     CTTACCCAAC GTAGCTAATG CATACGGCCT AGCTGATTGA TATTGGGATC GCAGTTCCCG GCATAGTGAT ATTCAGCGCT GCTAAGGTTT TTGTGTAAGA
```

FIG. 9D

```
                                                                                  sau96I
                                                                                  avaII
                                                                                  asuI
                                                                               scrFI
                                                                               mvaI
                                                                               ecoRII
                                                                               dsaV
                                                                               bstNI                                    ecoRI
           maeIII                                                              apyI[dcm+]       mnlI        taqI    apoI
          hphI    scfI        fokI                                      bslI  bsaJI            bsaJI       claI/bsp106
1201 ATACGATTTA GGTGACACTA TAGATAACAT CCACTTTGCC TTTCTCTCCA CAGGTGTCCA CTCCCAGGTC CAACTGCACC TCGGTTCTAT CGATTGAATT
     TATGCTAAAT CCACTGTGAT ATCTATTGTA GGTGAAACGG AAAGAGAGGT GTCCACAGGT GAGGGTCCAG GTTGACGTGG AGCCAAGATA GCTAACTTAA

                                                                                                    scrFI
      nlaIII                                                                                        mvaI
     styI                                                                                           ecoRII
     pflMI                                                                                          dsaV
     ncoI                                                                                           bstNI fnu4HI
     dsaI                                                      rsaI            aluI                 apyI[dcm+]
     bslI fokI                          rmaI              gsuI/bpmI           pvuII     pleI       haeI     bbvI
     bsaJI       nlaIII  fokI           maeI             bsrI  csp6I          nspBII    hinfI   aciI haeIII/palI
1301 CCACCATGGG ATGGTCATGT ATCATCCTTT TTCTAGTAGC AACTGCAACT GGAGTACATT CAGAAGTTCA GCTGGTGGAG TCTGGCGGTG GCCTGGTGCA
     GGTGGTACCC TACCAGTACA TAGTAGGAAA AAGATCATCG TTGACGTTGA CCTCATGTAA GTCTTCAAGT CGACCACCTC AGACCGCCAC CGGACCACGT
```

FIG. 9C

```
                                                                                                haeIII/palI
                                                                                               haeI
                                                                       scrFI          scrFI
                                                                       mvaI                 mvaI
                                                                       ecoRII         ecoRII
         mspI                                                          dsaV   tfiI    dsaV
         hpaII                                                         bstNI    nlaIII      bstNI     ddeI pleI
        bsaWI                accI nlaIII             mnlI              apyI[dcm+]    hinfI  apyI[dcm+]     hinfI
 801 ACAACCGGAA TTGGCAAGTA AAGTAGACAT GGTTTGGATA GTCGGAGGCA GTTCTGTTTA CCAGGAAGCC ATGAATCAAC CAGGCCACCT TAGACTCTTT
     TGTTGGCCTT AACCGTTCAT TTCATCTGTA CCAAACCTAT CAGCCTCCGT CAAGACAAAT GGTCCTTCGG TACTTAGTTG GTCCGGTGGA ATCTGAGAAA

                                                                                                      hgaI
                                                                                                     hinlI/acyI
                                                                                                     ahaII/bsaHI
                                                                                                  scrFI
                 nlaIII                                                                           mvaI     mnlI
             sau3AI                                                                               ecoRII
             mboI/ndeII[dam-]                                                                     dsaV
             dpnI[dam+]                  maeII                                                    bstNI    ecoNI
             dpnII[dam-]               aflIII                                                     apyI[dcm+]      mnlI
     maeIII alwI[dam-]  apoI       maeIII                                        mnlI            bsaJI     bslI ddeI
 901 GTGACAAGGA TCATGCAGGA ATTTGAAAGT GACACGTTTT TCCCAGAAAT TGATTTGGGG AAATATAAAC CTCTCCCAGA ATACCCAGGC GTCCTCTCTG
     CACTGTTCCT AGTACGTCCT TAAACTTTCA CTGTGCAAAA AGGGTCTTTA ACTAAACCCC TTTATATTTG GAGAGGGTCT TATGGGTCCG CAGGAGAGAC

         scrFI
         mvaI
         ecoRII
         dsaV
         bstNI
         apyI[dcm+]
      sau96I
      avaII                                                           sfaNI
      asuI   mnlI       sfaNI                 accI     mboII             mboII                   mnlI    aluI
1001 AGGTCCAGGA GGAAAAAGGC ATCAAGTATA AGTTTGAAGT CTACGAGAAG AAAGACTAAC AGGAAGATGC TTTCAAGTTC TCTGCTCCCC TCCTAAAGCT
     TCCAGGTCCT CCTTTTTCCG TAGTTCATAT TCAAACTTCA GATGCTCTTC TTTCTGATTG TCCTTCTACG AAAGTTCAAG AGACGAGGGG AGGATTTCGA
                                                     styI
                                                     bsaJI
                                               sau3AI
                       nlaIII                  mboI/ndeII[dam-]
                      styI                     dpnI[dam+]
                      ncoI                     dpnII[dam-]                aluI      tru9I
     ppu10I           dsaI                     alwI[dam-]                fnu4HI     mseI
     nsiI/avaIII      bsaJI                   bstYI/xhoII                bbvI      aseI/asnI/vspI
1101 ATGCATTTTT ATAAGACCAT GGGACTTTTG CTGGCTTTAG ATCCCCTTGG CTTCGTTAGA ACGCAGCTAC AATTAATACA TAACCTTATG TATCATACAC
     TACGTAAAAA TATTCTGGTA CCCTGAAAAC GACCGAAATC TAGGGGAACC GAAGCAATCT TGCGTCGATG TTAATTATGT ATTGGAATAC ATAGTATGTG
```

FIG. 9B

```
                              tfiI
    scrFI                     hinfI
    nciI                   aciI
    mspI                  thaI                                                                    fnu4HI
    hpaII                 fnuDII/mvnI                         aciI                                bbvI
    dsaV                  bstUI                     maeII  rsaI                                 nspBII
    cauII                 bsh1236I               maeIII    csp6I  scfI            mnlI          aciI       nlaIII
401 CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG AGTGACGTAA GTACCGCCTA TAGAGCGATA AGAGGATTTT ATCCCCGCTG CCATCATGGT
    GGCCCTTGCC ACGTAACCTT GCGCCTAAGG GGCACGGTTC TCACTGCATT CATGGCGGAT ATCTCGCTAT TCTCCTAAAA TAGGGGCGAC GGTAGTACCA

                                                                              haeIII/palI
                                                                             haeI
                                                                           scrFI
                                                                           mvaI     bsrBI
                                                                           ecoRII
                                                                           dsaV
                                                                           bstNI    aciI                     rsaI
                                     pflMI                       bsmAI     apyI[dcm+]        xmnI            csp6I
    taqI   .       sfaNI             bslI                        bsaI     bsaJI  mnlI  ddeI  asp700         scaI
501 TCGACCATTG AACTGCATCG TCGCCGTGTC CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT CAAGTACTTC
    AGCTGGTAAC TTGACGTAGC AGCGGCACAG GGTTTTATAC CCCTAACCGT TCTTGCCTCT GGATGGGACC GGAGGCGAGT CCTTGCTCAA GTTCATGAAG

                                                                          scrFI
                                                                          mvaI
                                                                          ecoRII
                        eco57I                                            dsaV
                       mboII                 tfiI                         bstNI                   tfiI       tru9I
                      earI/ksp632I           hinfI                        apyI[dcm+]              hinfI      mseI
                     mnlI                  alwNI    hphI                 sexAI            ddeI mboII taqI   ahaIII/draI
601 CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA
    GTTTCTTACT GGTGTTGGAG AAGTCACCTT CCATTTGTCT TAGACCACTA ATACCCATCC TTTTGGACCA AGAGGTAAGG ACTCTTCTTA GCTGGAAATT

                                                            sstI
                                                            sacI
                                                            hgiJII
                                                            hgiAI/aspHI
                                                            ecl136II
                                                            bsp1286
                                                            bsiHKAI
             tru9I                                          bmyI                                  tru9I
             mseI         ddeI                              banII                              . aflII/bfrI
            aseI/asnI/vspI                       bslI   mnlI aluI           bstXI      fokI sfaNI mseI
701 AGGACAGAAT TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA AAGTTTGGAT GATGCCTTAA GACTTATTGA
    TCCTGTCTTA ATTATATCAA GAGTCATCTC TTGAGTTTCT TGGTGGTGCT CCTCGAGTAA AAGAACGGTT TTCAAACCTA CTACGGAATT CTGAATAACT
```

FIG. 9A

```
     aluI
    sstI
    sacI                                       aluI
    hgiJII                               sau3AI pvuII                                                   nlaIV
    hgiAI/aspHI                          mboI/ndeII[dam-]                                             scrFI
    ecl136II                             dpnI[dam+]                                                   mvaI
    bsp1286                             pvuI/bspCI                                                    ecoRII
    bsiHKAI                          pleI dpnII[dam-]                                                 dsaV
    bmyI                             hinfI  taqI[dam-]                                                bstNI
    banII                         rmaI    mcrI    nspBII                                              apyI[dcm+]
   taqI                           maeI   taqI[dam-]                                                  bsaJI
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGAGTCGATC GACAGCTGTG GAATGTGTGT CAGTTAGGGT GTGGAAAGTC CCCAGGCTCC CCAGCAGGCA
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCTCAGCTAG CTGTCGACAC CTTACACACA GTCAATCCCA CACCTTTCAG GGGTCCGAGG GGTCGTCCGT

                                                       nlaIV
             sfaNI                 scrFI         scrFI                              sfaNI
            ppu10I                 mvaI              mvaI                                   ppu10I
            nsiI/avaIII            ecoRII        ecoRII                           nsiI/avaIII
           nlaIII                  dsaV          dsaV                             nlaIII
          sphI                     bstNI             bstNI                                sphI
          nspI                     apyI[dcm+]        apyI[dcm+]                           nspI
          nspHI                   sexAI             bsaJI                                 nspHI
101 GAAGTATGCA AAGCATGCAT CTCAATTAGT CAGCAACCAG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC ATCTCAATTA
    CTTCATACGT TTCGTACGTA GAGTTAATCA GTCGTTGGTC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG TAGAGTTAAT

                                                                                    nlaIII
                                                                                   styI
                                                                                   ncoI
                                  aciI                                         bslI dsaI
             aciI         aciI  fokI               aciI bsrI  aciI             aciI bsaJI
201 GTCAGCAACC ATAGTCCCGC CCCTAACTCC GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG GCTGACTAAT TTTTTTTATT
    CAGTCGTTGG TATCAGGGCG GGGATTGAGG CGGGTAGGGC GGGGATTGAG GCGGGTCAAG GCGGGTAAGA GGCGGGGTAC CGACTGATTA AAAAAAATAA

                                                                           rmaI
                                                                          styI
             fnu4HI                                                       bsaJI                     haeIII/palI
             bglI                                                         blnI                     mcrI
            sfiI                                                          avrII          aluI      eagI/xmaIII/eclXI
            haeIII/palI                                                 haeIII/palI     rmaI       eaeI
          mnlI   mnlI       ddeI                                        stuI            maeI       cfrI
      haeIII/palI bsaJI  mnlI    aluI                      mnlI         haeI           nheI       mspI
    mnlI bsaJI aciI   haeIII/palI                      mnlI           mnlI maeI      aluI         hpaII
301 TATGCAGAGG CCGAGGCCGC CTCGGCCTCT GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG CAAAAAGCTA GCTTATCCGG
    ATACGTCTCC GGCTCCGGCG GAGCCGGAGA CTCGATAAGG TCTTCATCAC TCCTCCGAAA AAACCTCCGG ATCCGAAAAC GTTTTTCGAT CGAATAGGCC
```

FIG. 8

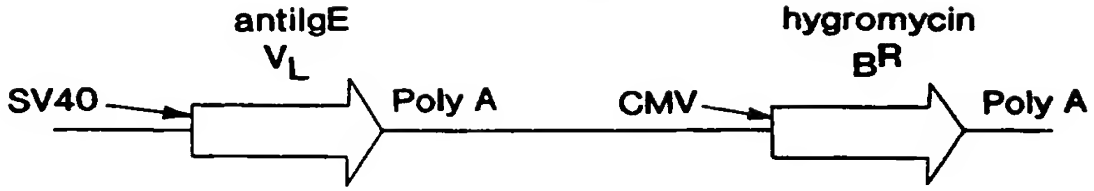
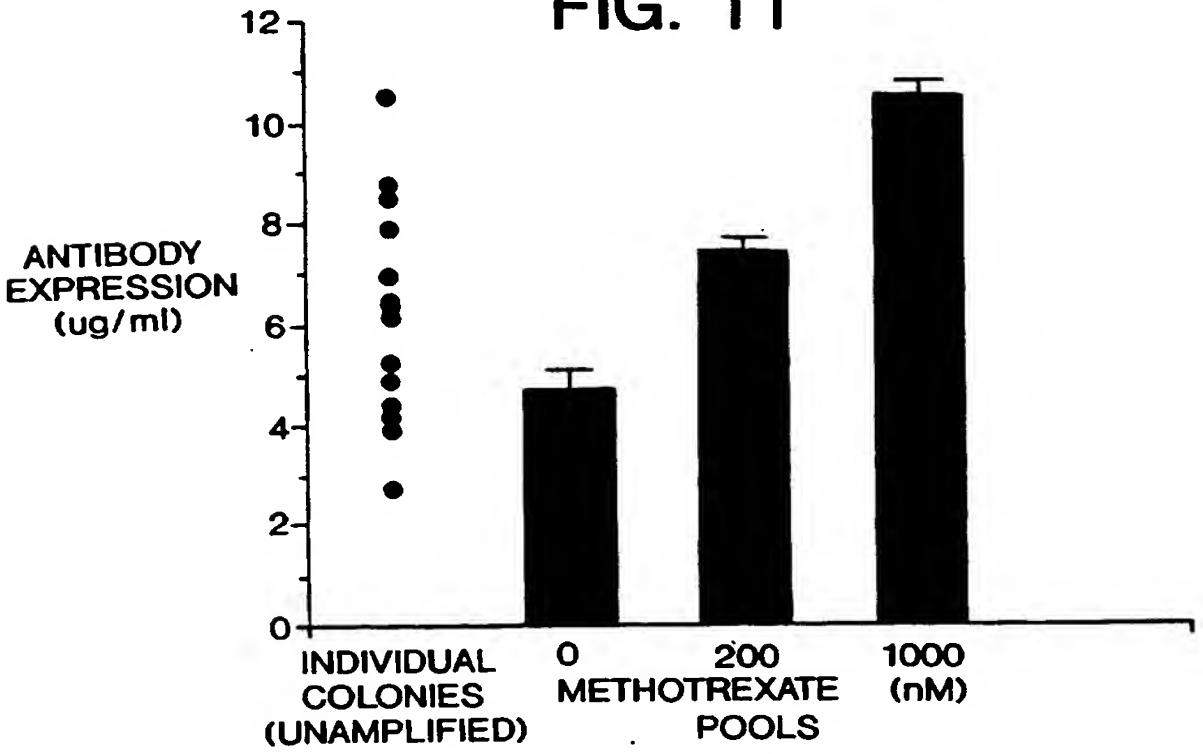


FIG. 11



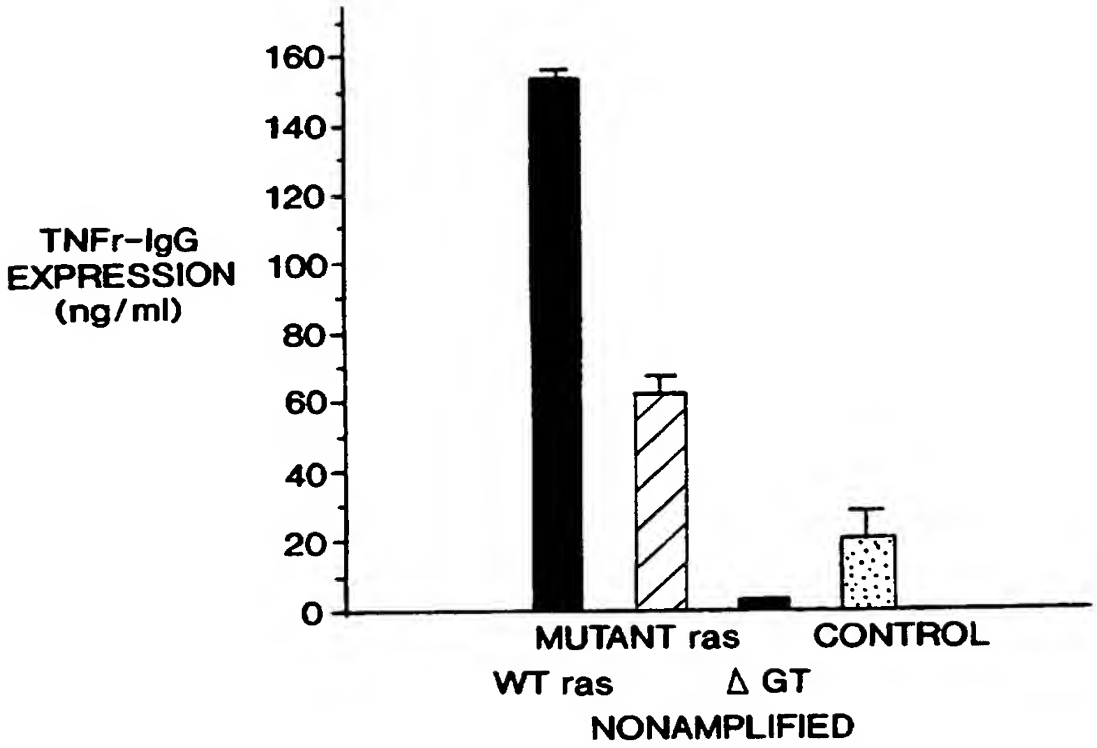


FIG. 7A

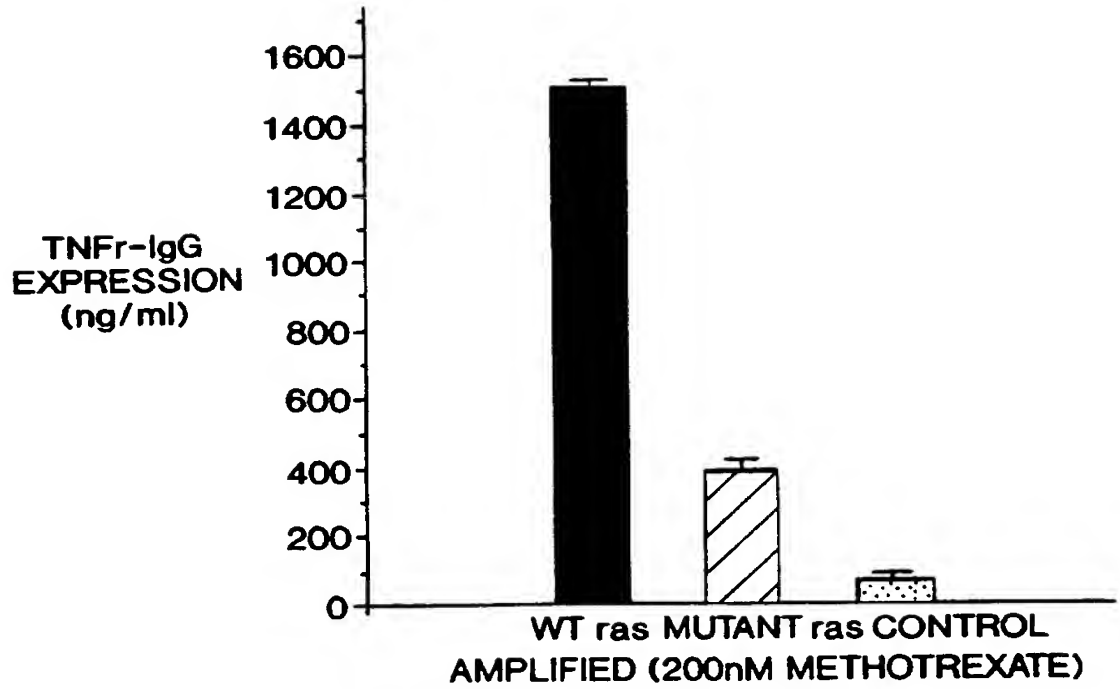


FIG. 7B

FIG. 6R

```
  haeIII/palI
 haeI
scrFI
mvaI bslI
ecoRII                      nlaIII
dsaV                       nspI
bstNI                haeIII/palI  nspHI                    tfiI                                         bsrBI
apyI[dcm+]            haeI        aflIII                   hinfI                aciI        aluI        aciI
6501 CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC TGATACCGCT
     GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG ACTATGGCGA

                                                                                             thaI
                                                                                             fnuDII/mvnI
                                                                                             bstUI
                                                                                             bsh1236I
                                                                                            hinPI
                                                                                            hhaI/cfoI
                                                                                           thaI
                                                                                           fnuDII/mvnI
   fnu4HI               fnu4HI                                sapI  hinPI                   bstUI   haeIII/palI
   bbvI                 bbvI pleI                             mboII hhaI/cfoI               bsh1236I          tru9I
  aciI                 hinPI  hinfI                           earI/ksp632I          mnlI    bslI     eaeI  tfiI aseI/asnI/vspI
 fnu4HI      mcrI      hhaI/cfoI         mnlI    aciI         haeII             aciI        aciI     cfrI  hinfI mseI
6601 CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG CCGATTCATT
     GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC GGCTAAGTAA

                                                                                                   scrFI
                                                                                                   mvaI
                                                                                                   ecoRII
                                                                                                   dsaV
    aluI                                                         tru9I                        nlaIV bstNI
   pvuII                                              hinPI      mseI     maeIII              hgiCI apyI[dcm+]
   nspBII                    bsrI     aciI            hhaI/cfoI  aseI/asnI/vspI  mnlI         banI bsaJI
6701 AATCCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG CAGTGAGCGC AACGCAATTA ATGTGAGTTA CCTCACTCAT TAGGCACCCC AGGCTTTACA
     TTAGGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC GTCACTCGCG TTGCGTTAAT TACACTCAAT GGAGTGAGTA ATCCGTGGGG TCCGAAATGT

                                                                                              tru9I
                                                                                              mseI
                                                                                             aseI/asnI/vspI
              mspI                     aciI                                                  xmnI
              hpaII                   bsrBI                             aluI       nlaIII    asp700
6801 CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTAA
     GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAATT

>length: 6889
```

FIG. 6Q

```
                                                    scrFI                                               aciI
                                                    nciI                                              nspBII
                      fnu4HI                        mspI                               mspI          fnu4HI
          alwNI       bbvI                          hpaII                              hpaII         bbvI
         bsrI   fnu4HI                              dsaV      pleI                    bsaWI        hinPI mcrI
     maeIII     bbvI      bsrI                      cauII     hinfI                maeIII          hhaI/cfoI
6101 TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC GGTCGGGCTG
     ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG CCAGCCCGAC

           hgiAI/aspHI
           bsp1286
           bsiHKAI
           bmyI                                                                                       hinPI
           apaLI/snoI                                                                                 hhaI/cfoI
           alw44I/snoI   aluI                                 ddeI       scfI                       haeII
6201 AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG ACCTACACCG AACTGAGATA CCTACAGCGT GAGCATTGAG AAAGCGCCAC GCTTCCCGAA
     TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGTAACTC TTTCGCGGTG CGAAGGGCTT

                                                                            scrFI
                                                                            mvaI           scrFI
                                                                            ecoRII  mvaI
                            mspI                                            dsaV    ecoRII
                            hpaII                                           bstNI          dsaV
                            bslI  fnu4HI                       hinPI mnlI   bsaJI          bstNI
          aciI             bsaWI  aciI                         hhaI/cfoI  aluI apyI[dcm+]  apyI[dcm+]
6301 GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT TATAGTCCTG
     CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA ATATCAGGAC

                                                                                                 haeIII/palI
                                                                                               fnu4HI
                                                                                               aciI
                                                                                              thaI bslI
                                                                                              fnuDII/mvnI
                                 taqI                              nlaIV                      bstUI
             mnlI drdI       hgaI            sfaNI               aciI                         bsh1236I    nlaIV
6401 TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT TTTTACGGTT
     AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA AAAATGCCAA
```

FIG. 6P

```
                                                                                                                      rmaI      sau3AI
                                                                                                                 sau3AI hphI    mboI/ndeII[dam-]
                                                                                                                 mboI/ndeII[dam-]
                                                                                                                 dpnI[dam+]     dpnI[dam+]
                                                                                                                 dpnII[dam-]    dpnII[dam-]
                                                                       tru9I                          ahaIII/draI    maeI       alwI[dam-]
                                                                       mseI                     tru9I  tru9I bstYI/xhoII    bstYI/xhoII
             maeIII                                                    ahaIII/draI              mseI   mseI  alwI[dam-]    mboII[dam-]
5701 AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG ATCCTTTTTG
     TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC TAGGAAAAAC

                                                                                                             sau3AI
                                                                                                             mboI/ndeII[dam-]
                                                                                                             dpnI[dam+]    sau3AI
                                                                                                             dpnII[dam-]   mboI/ndeII[dam-]
                                                                                                            bstYI/xhoII    dpnI[dam+]
                                                                                                    sau3AI  alwI[dam-]     dpnII[dam-]
            nlaIII                 maeII                                                            mboI/ndeII[dam-]       alwI[dam-]
           rcaI                 tru9I                         hgaI                                  dpnI[dam+] mboII[dam-]
           bspHI                mseI                      ddeI                                      dpnII[dam-]          bstYI/xhoII
5801 ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC CTTTTTTTCT
     TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG GAAAAAAAGA

                                                                                       sau3AI
      thaI                                                                             mboI/ndeII[dam-]
      fnuDII/mvnI                                                                      dpnI[dam+]
      bstUI                                                                            dpnII[dam-]
      bsh1236I                                                                        alwI[dam-]
     hinPI       fnu4HI                                     aciI                  mspI                                        bsrI
     hhaI/cfoI   bbvI                            aciI    nspBII                   hpaII      aluI                        maeIII    eco57I
5901 GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA GGTAACTGGC
     CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT CCATTGACCG

             hinPI                               rmaI                 haeIII/palI
             hhaI/cfoI                           maeI      bslI       haeI                     scfI     aciI         mnlI
6001 TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC CTCGCTCTGC
     AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG GAGCGAGACG
```

FIG. 6O

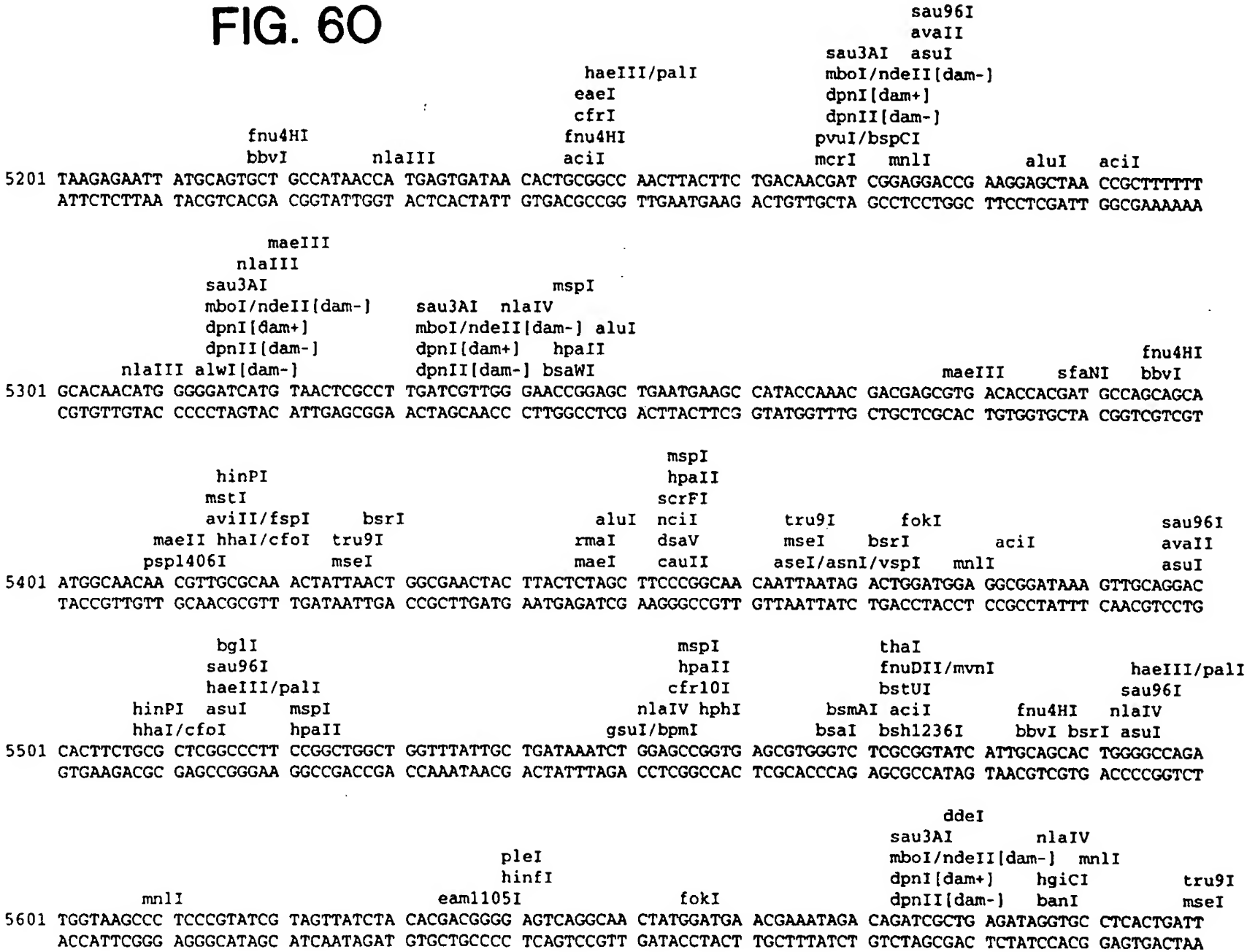


FIG. 6N

```
                                                              nlaIV
                                                            aciI
                                                           thaI
                                                           fnuDII/mvnI
                          hinlI/acyI                       bstUI                                                       rcaI
                          ahaII/bsaHI                      bsh1236I                                                    bspHI
                          aatII                           hinPI                                                    bsrBI
                      ddeI maeII                          hhaI/cfoI                                                aciI nlaIII
4701 AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT GTATCCGCTC
     TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA CATAGGCGAG

                                                         mboII                                                     fnu4HI
      bsmAI                               sspI           earI/ksp632I                                              aciI
4801 ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT TTTGCGGCAT
     TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA AAACGCCGTA

                                                                              hgiAI/aspHI
                                                                              bsp1286
                                                                   sau3AI     bsiHKAI                             sau3AI
                                                                   mboI/ndeII(dam-)                               mboI/ndeII(dam-)
                                                                   dpnI(dam+) bmyI                                dpnI(dam+)
                                                                   dpnII(dam-)                                   bstYI/xhoII
                                                              eco57I          apaLI/snoI                     bsrI dpnII(dam-)
                             hphI            hphI         sfaNI  mboII(dam-)  alw44I/snoI  maeIII taqI       alwI(dam-)
4901 TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC TGGATCTCAA
     AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG ACCTAGAGTT

                                                                                          aciI
                  sau3AI                                                                 thaI
                  mboI/ndeII(dam-)                maeII                                  fnuDII/mvnI
                  dpnI(dam+)                     psp1406I           hgiAI/aspHI          bstUI
                  dpnII(dam-)                   xmnI                bsp1286 tru9I        bsh1236I             hinlI/acyI
         aciI     alwI(dam-)                    asp700              bsiHKAI mseI        hinPI                hgaI
     nspBII  bstYI/xhoII                     mboII                  bmyI   ahaIII/draI  hhaI/cfoI            ahaII/bsaHI
5001 CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC CCGTGATGAC
     GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG GGCACTACTG

      scrFI
      nciI
      mspI
      hpaII                                                        rsaI
      dsaV                      aciI                               csp6I   bsrI
      cauII      bcgI  mcrI   fnu4HI          ddeI                scaI  hphI  maeIII      sfaNI     fokI    nlaIII
5101 GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT GGCATGACAG
     CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA CCGTACTGTC
```

FIG. 6M

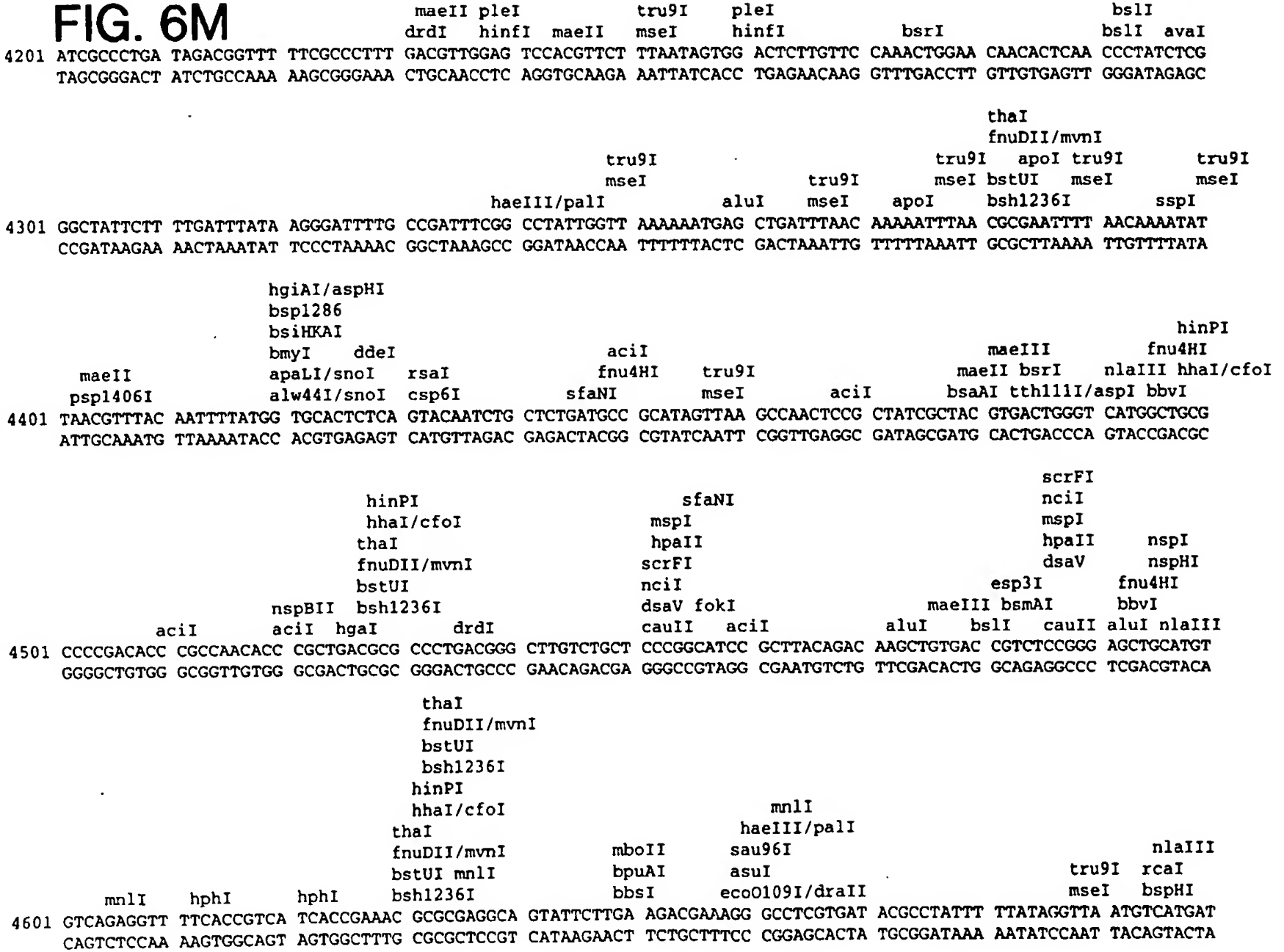


FIG. 6L

```
                                                                                                               hinPI
                                                                                                               hhaI/cfoI
                                                                                                              nlaIV
                                                         sau3AI                                               narI
                                             sau96I      mboI/ndeII[dam-]                                     kasI
                                             haeIII/palI                                                      hinlI/acyI
                                             asuI        dpnI[dam+]                                           hgiCI
                 aluI                      mnlI          dpnII[dam-]                                          haeII     aciI
                 pvuII                  mboII   aciI    pvuI/bspCI                                            banI   sfaNI
     fokI        nspBII                 earI/ksp632I    mcrI                               bglI               ahaII/bsaHI
3801 CATCCCCCCT TCGCCAGCTG GCGTAATAGC GAAGAGGCCC GCACCGATCG CCCTTCCCAA CAGTTGCGTA GCCTGAATGG CGAATGGCGC CTGATGCGGT
     GTAGGGGGGA AGCGGTCGAC CGCATTATCG CTTCTCCGGG CGTGGCTAGC GGGAAGGGTT GTCAACGCAT CGGACTTACC GCTTACCGCG GACTACGCCA

                                                                                                        aciI
                                                                                                      fnu4HI
                                                                              hinPI                   aciI
                                                                             thaI                    thaI
                                                                             fnuDII/mvnI             fnuDII/mvnI
                                                                             bstUI scfI    hinPI     bstUI
                                                                             bsh1236I      hhaI/cfoI hinPI
                                                                         rsaI hhaI/cfoI  fnu4HI  tru9I bsh1236I
                 sfaNI     aciI          aciI    maeII                   csp6I    bslI   aciI    mseI hhaI/cfoI
3901 ATTTTCTCCT TACGCATCTG TGCGGTATTT CACACCGCAT ACGTCAAAGC AACCATAGTA CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG
     TAAAAGAGGA ATGCGTAGAC ACGCCATAAA GTGTGGCGTA TGCAGTTTCG TTGGTATCAT GCGCGGGACA TCGCCGCGTA ATTCGCGCCG CCCACACCAC

          fnu4HI
        hinPI
        hhaI/cfoI                                  hinPI
       thaI                                        hhaI/cfoI
       fnuDII/mvnI                               rmaI                                                   mspI
       bstUI                                 hinPI  haeII                                               hpaII
       bsh1236I     aciI                     hhaI/cfoI    bsrBI                                        naeI
     maeIII bbvI  maeIII                    haeII maeI    aciI           mboII               maeII  cfr10I
4001 GTTACGCGCA GCGTGACCGC TACACTTGCC AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCCT TTCTCGCCAC GTTCGCCGGC TTTCCCCGTC
     CAATGCGCGT CGCACTGGCG ATGTGAACGG TCGCGGGATC GCGGGCGAGG AAAGCGAAAG AAGGGAAGGA AAGAGCGGTG CAAGCGGCCG AAAGGGGCAG

                 nlaIV
                hgiJII
                bsp1286                                  nlaIV                                    maeII    haeIII/palI
                bmyI                                     hgiCI  taqI                              draIII  sau96I
      aluI      banII         nlaIV                      banI mnlI                 hphI           bsaAI   asuI
4101 AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCCGATTTAG TGCTTTACGG CACCTCGACC CCAAAAAACT TGATTTGGGT GATGGTTCAC GTAGTGGGCC
     TTCGAGATTT AGCCCCCGAG GGAAATCCCA AGGCTAAATC ACGAAATGCC GTGGAGCTGG GGTTTTTTGA ACTAAACCCA CTACCAAGTG CATCACCCGG
```

FIG. 6K

```
                 nlaIV                                                                          nlaIV
             scrFI                                                  scrFI        scrFI
             mvaI                                 ppu10I                  mvaI               mvaI
             ecoRII                         nsiI/avaIII             ecoRII       ecoRII
             dsaV                          nlaIII                   dsaV         dsaV
             bstNI                             sphI                       bstNI              bstNI
             apyI[dcm+]                        nspI sfaNI                 apyI[dcm+]         apyI[dcm+]
            bsaJI                              nspHI                     sexAI             bsaJI
3401 GAAAGTCCCC AGGCTCCCCA GCAGGCAGAA GTATGCAAAG CATGCATCTC AATTAGTCAG CAACCAGGTG TGGAAAGTCC CCAGGCTCCC CAGCAGGCAG
     CTTTCAGGGG TCCGAGGGGT CGTCCGTCTT CATACGTTTC GTACGTAGAG TTAATCAGTC GTTGGTCCAC ACCTTTCAGG GGTCCGAGGG GTCGTCCGTC

                     sfaNI
                   ppu10I
                   nsiI/avaIII
                  nlaIII
                 sphI
                 nspI                                                       aciI                               bslI
                 nspHI                              aciI         aciI   fokI             aciI bsrI  aciI        aciI
3501 AAGTATGCAA AGCATGCATC TCAATTAGTC AGCAACCATA GTCCCGCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC CCAGTTCCGC CCATTCTCCG
     TTCATACGTT TCGTACGTAG AGTTAATCAG TCGTTGGTAT CAGGGCGGGG ATTGAGGCGG GTAGGGCGGG GATTGAGGCG GGTCAAGGCG GGTAAGAGGC

                                                                                                               rmaI
                                                                                                              styI
                                                                                                              bsaJI
                                                    fnu4HI                                                    blnI
        nlaIII                                     sfiI mnlI                                                  avrII
       styI                                        haeIII/palI                                              haeIII/palI
       ncoI                                  bsaJI bglI           ddeI                                     stuI
       dsaI                                haeIII/palI bsaJI   mnlI  aluI                   mnlI           haeI
       bsaJI                             mnlI  mnlI aciI    haeIII/palI                  mnlI             mnlI maeI
3601 CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG
     GGGGTACCGA CTGATTAAAA AAAATAAATA CGTCTCCGGC TCCGGCGGAG CCGGAGACTC GATAAGGTCT TCATCACTCC TCCGAAAAAA CCTCCGGATC

                                                                               scrFI
                                                                               mvaI
                                                                               ecoRII
                        tru9I             haeIII/palI                          dsaV
                       hpaI              eaeI                                  bstNI
                       hincII/hindII     cfrI                  bsrI            apyI[dcm+]       tru9I       fnu4HI
                   aluI mseI  aluI     bsrI               maeII maeIII        bsaJI     maeIII   mseI        bbvI
3701 GCTTTTGCAA AAAGCTGTTA ACAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG GGAAAACCCT GGCGTTACCC AACTTAATCG CCTTGCAGCA
     CGAAAACGTT TTTCGACAAT TGTCGAACCG TGACCGGCAG CAAAATGTTG CAGCACTGAC CCTTTTGGGA CCGCAATGGG TTGAATTAGC GGAACGTCGT
```

FIG. 6J

```
          rmaI
        mnlI
      sau3AI
      mboI/ndeII[dam-]                    styI
      dpnI[dam+]                        aciI
      dpnII[dam-]  taqI                fnu4HI   sau96I
      alwI[dam-]  salI   scfI         sfiI ncoI haeIII/palI
     nlaIV  maeI  hincII/hindII       haeIII/palI
     bstYI/xhoII  accI   pstI        eaeI  dsaI asuI              aluI
     bamHI xbaI pleI     bsgI   aluI cfrI  bsaJI               fnu4HI
     alwI[dam-] hinfI bspMI    hindIII bglI nlaIII              bbvI          maeIII            sfaNI   apoI
3101 GGGGATCCTC TAGAGTCGAC CTGCAGAAGC TTGGCCGCCA TGGCCCAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT
     CCCCTAGGAG ATCTCAGCTG GACGTCTTCG AACCGGCGGT ACCGGGTTGA ACAAATAACG TCGAATATTA CCAATGTTTA TTTCGTTATC GTAGTGTTTA

                                                                                                    sau3AI
                                                                                                    mboI/ndeII[dam-]
                                                                                                    dpnI[dam+]
                                                                                                    dpnII[dam-]
                                                                                                   pvuI/bspCI
                                                                                                   mcrI
                                                                                                  taqI[dam-]   tru9I
                                                                                                 claI/bsp106[dam-]
                                                                                                sau3AI         mseI
                                                                                                mboI/ndeII[dam-]
                                                                                                dpnI[dam+] xmnI        hinPI
                                        rmaI                                                    dpnII[dam-]  aseI/asnI/vspI
                                  bsmI  maeI                                          nlaIII  alwI[dam-]  asp700      hhaI/cfoI
3201 TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CGATCGGGAA TTAATTCGGC
     AAGTGTTTAT TTCGTAAAAA AAGTGACGTA AGATCAACAC CAAACAGGTT TGAGTAGTTA CATAGAATAG TACAGACCTA GCTAGCCCTT AATTAAGCCG

                                                rsaI
                                                csp6I
          haeI                                nlaIV
        styI                                  kpnI
        ncoI                                  hgiCI
        dsaI haeIII/palI                      banI                      aluI
     fnu4HI nlaIII                            asp718     mnlI          pvuII
     bbvI  bsaJI             mnlI      mnlI   acc65I   ddeI aciI       nspBII
3301 GCAGCACCAT GGCCTGAAAT AACCTCTGAA AGAGGAACTT GGTTAGGTAC CTTCTGAGGC GGAAAGAACC AGCTGTGGAA TGTGTGTCAG TTAGGGTGTG
     CGTCGTGGTA CCGGACTTTA TTGGAGACTT TCTCCTTGAA CCAATCCATG GAAGACTCCG CCTTTCTTGG TCGACACCTT ACACACAGTC AATCCCACAC
```

FIG. 6I

```
                                                                                                      rsaI
                 bsmAI                                                                                csp6I
                bsaI            mnlI           taqI                              avaI            bsp1407I    bslI
2701 ACAAGTGCAA GGTCTCCAAC AAAGCCCTCC CAGCCCCCAT CGAGAAAACC ATCTCCAAAA CCAAAGGACA GCCCCGAGAA CCACAGGTGT ACACCCTGCC
     TGTTCACGTT CCAGAGGTTG TTTCGGGAGG GTCGGGGGTA GCTCTTTTGG TAGAGGTTTT GGTTTCCTGT CGGGGCTCTT GGTGTCCACA TGTGGGACGG

           scrFI
           nciI
           mspI
           hpaII
           dsaV
           cauII
          xmaI/pspAI
          smaI
          scrFI                   scrFI         scrFI
          nciI                    mvaI                mvaI
          dsaV                    ecoRII        ecoRII
          cauII                   dsaV          dsaV                                              aciI
          bsaJI                   bstNI               bstNI                             dsaI              nspBII
       fokI                       apyI[dcm+]          apyI[dcm+]                        bslI             fnu4HI fnu4HI
     bslI avaI  mnlI             sexAI           bspMI                                  bsaJI            bbvI   bbvI
2801 CCCATCCCGG GAGGAGATGA CCAAGAACCA GGTCAGCCTG ACCTGCCTGG TCAAAGGCTT CTACCCCAGC GACATCGCCG TGGAGTGGGA GAGCAGCGGG
     GGGTAGGGCC CTCCTCTACT GGTTCTTGGT CCAGTCGGAC TGGACGGACC AGTTTCCGAA GATGGGGTCG CTGTAGCGGC ACCTCACCCT CTCGTCGCCC

                                                                                         dsaI
        mspI                                   pleI                 mnlI            hphI                    fnu4HI
        hpaII                    mnlI  nlaIII  hinfI     nlaIV   mboII    scfI     aluI  bsaJI        bspMI bbvI
2901 CAGCCGGAGA ACAACTACAA CACCACGCCT CCCATGCTGG ACTCCGACGG CTCCTTCTTC CTCTACAGCA AGCTCACCGT GGACAAGAGC AGGTGGCAGC
     GTCGGCCTCT TGTTGATGTT GTGGTGCGGA GGGTACGACC TGAGGCTGCC GAGGAAGAAG GAGATGTCGT TCGAGTGGCA CCTGTTCTCG TCCACCGTCG

                                                                                                            scrFI
                                                                                                            nciI
                                                                                                            mspI
                                                                                                            hpaII
                                                                                                            dsaV
                                                                                          scrFI             cauII
                                                                                          nciI              bsaJI
                                                                                          mspI            haeIII/palI
                                    nlaIII                                                hpaII          mcrI
                                 ppu10I                            sapI                   dsaV           eagI/xmaIII/eclXI
         xmnI mboII              nsiI/avaIII                       mboII  mnlI       bsmAI               eaeI
         asp700     nlaIII       sfaNI   mnlI          aciI        earI/ksp632I bslI      cauII          cfrI
3001 AGGGGAACAT CTTCTCATGC TCCGTGATGC ATGAGGCTCT GCACAACCGC TTCACGCAGA AGAGCCTCTC CCTGTCTCCG GGTAAATGAG TGCGACGGCC
     TCCCCTTGTA GAAGAGTACG AGGCACTACG TACTCCGAGA CGTGTTGGCG AAGTGCGTCT TCTCGGAGAG GGACAGAGGC CCATTTACTC ACGCTGCCGG
```

FIG. 6H

```
                                                               eam1105I
                                                              sau96I
                                                       scrFI
                                     bsp1286           mvaI    avaII
                                    nlaIV              ecoRII
                                    hgiCI              dsaV
                                dsaI bmyI              bstNI   asuI        mboII mboII
                                bsaJI                  bsaJI mnlI         bpuAI earI/ksp632I     styI
        maeIII   mnlI   nlaIII  banI   alwNI           apyI[dcm+]         bbsI mnlI              bsaJI
2401 CTTGTGACAC ACCTCCCCCA TGCCCACGGT GCCCAGCACC TGAACTCCTG GGAGGACCGT CAGTCTTCCT CTTCCCCCCA AAACCCAAGG ATACCCTTAT
     GAACACTGTG TGGAGGGGGT ACGGGTGCCA CGGGTCGTGG ACTTGAGGAC CCTCCTGGCA GTCAGAAGGA GAAGGGGGGT TTTGGGTTCC TATGGGAATA

           sau96I
           nlaIV
           avaII
           asuI          maeII
          mspI          pmlI
          hpaII         eco72I                                     sau96I
         scrFI     mnlI bsaAI                            drdI      avaII             maeII
         nciI    ddeI maeIII                           mboII    mnlI               bsaAI
         dsaV    eco81I  bbrPI                         bpuAI  bsaJI  bsrI          rsaI
         cauII   bsu36I/mstII/sauI          maeII      bbsI  avaI  asuI            csp6I         mnlI
2501 GATTTCCCGG ACCCCTGAGG TCACGTGCGT GGTGGTGGAC GTGAGCCACG AAGACCCCGA GGTCCAGTTC AAGTGGTACG TGGACGGCGT GGAGGTGCAT
     CTAAAGGGCC TGGGGACTCC AGTGCACGCA CCACCACCTG CACTCGGTGC TTCTGGGGCT CCAGGTCAAG TTCACCATGC ACCTGCCGCA CCTCCACGTA

                 aciI
                thaI
                fnuDII/mvnI
                bstUI
                bsh1236I
               sacII/sstII
               nspBII                                                          scrFI
               kspI                                                            mvaI bsrI
               dsaI                                                            ecoRII
               bsaJI                                                           dsaV
               aciI                                             mnlI     econI bstNI              rsaI
              fnu4HI  mnlI           maeII               hgaI   hphI   bslI   apyI[dcm+]          csp6I
2601 AATGCCAAGA CAAAGCCGCG GGAGGAGCAG TTCAACAGCA CGTTCCGTGT GGTCAGCGTC CTCACCGTCC TGCACCAGGA CTGGCTGAAC GGCAAGGAGT
     TTACGGTTCT GTTTCGGCGC CCTCCTCGTC AAGTTGTCGT GCAAGGCACA CCAGTCGCAG GAGTGGCAGG ACGTGGTCCT GACCGACTTG CCGTTCCTCA
```

FIG. 6G

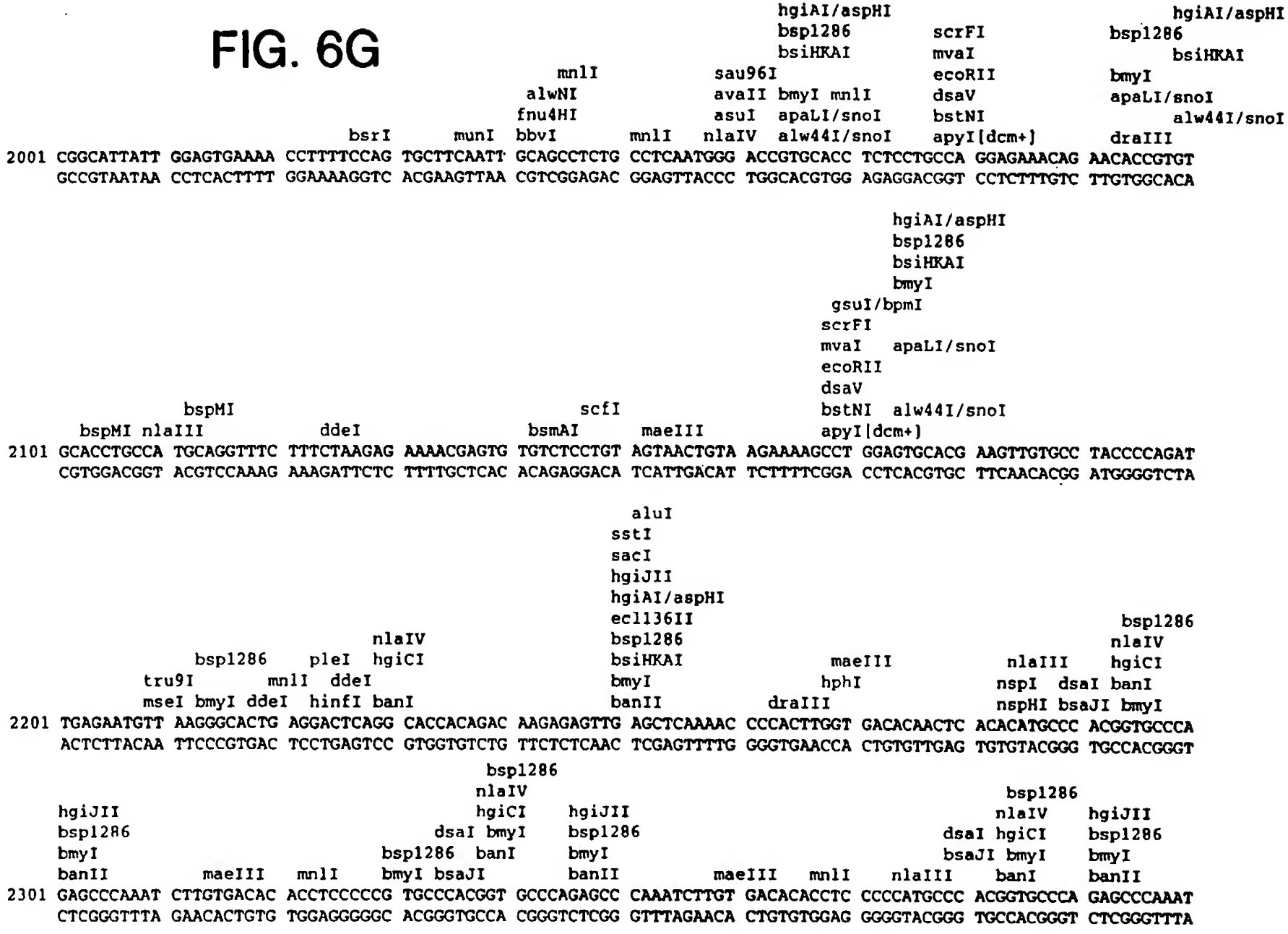


FIG. 6F

```
                                 scrFI
                                 nciI
                                 mspI
                                 hpaII
                                 dsaV
                                 cauII
                                xmaI/pspAI
                                smaI
                                scrFI
                                nciI
                                dsaV
                                cauII
                                bslI
                              sau96I
                              haeIII/palI
                              asuI
                           scrFI
                           mvaI bsaJI
                           ecoRII
                           dsaV                                   nlaIV
            rsaI           bstNI bsaJI          scfI                  fnu4HI                               alwNI
            csp6I          bslI avaI            pstI                  aciI         aciI                  ddeI
           bsp1407I        apyI[dcm+]           bsgI                bsrBI      hphI   eco57I           mnlI
1801 CCTACTTGTA CAATGACTGT CCAGGCCCGG GGCAGGATAC GGACTGCAGG GAGTGTGAGA GCGGCTCCTT CACCGCTTCA GAAAACCACC TCAGACACTG
     GGATGAACAT GTTACTGACA GGTCCGGGCC CCGTCCTATG CCTGACGTCC CTCACACTCT CGCCGAGGAA GTGGCGAAGT CTTTTGGTGG AGTCTGTGAC

                                                                        scrFI
                                                    mboII               nciI
                                                   earI/ksp632I         mspI
          fnu4HI                                 sau3AI                 hpaII
         aluI                                    mboI/ndeII[dam-]       dsaV                scfI            mspI
        pvuII                                    dpnI[dam+]             cauII               pstI            hpaII
        nspBII                                   dpnII[dam-]          sau96I                bsgI          rsaI
      ddeI                                      bstYI/xhoII           avaII                fnu4HI         csp6I
     mnlI bbvI                                  bglII                 asuI     draIII      bbvI   mboII  bsrI cfr10I
1901 CCTCAGCTGC TCCAAATGCC GAAAGGAAAT GGGTCAGGTG GAGATCTCTT CTTGCACAGT GGACCGGGAC ACCGTGTGTG GCTGCAGGAA GAACCAGTAC
     GGAGTCGACG AGGTTTACGG CTTTCCTTTA CCCAGTCCAC CTCTAGAGAA GAACGTGTCA CCTGGCCCTG TGGCACACAC CGACGTCCTT CTTGGTCATG
```

FIG. 6E

```
                                                                                                    haeIII/palI
                                                                                                   eaeI
                                                        scrFI                                0cfrI
                                                        mvaI                                      mspI
                                                        ecoRII                              hpaII
                                                        dsaV                               scrFI
                                                        bstNI                                    nciI
                                                        apyI[dcm+]      mnlI              ecoRI  dsaV
                                                      gsuI/bpmI         bsaJI       taqI  apoI   cauII
       scfI        fokI                           maeIII     hincII/hindII       claI/bsp106  bsaJI      aluI
1501 CACTATAGAA TAACATCCAC TTTGCCTTTC TCTCCACAGG TGTCACTCCA GGTCAACTGC ACCTCGGTTC TATCGATTGA ATTCCCCGGC CATAGCTGTC
     GTGATATCTT ATTGTAGGTG AAACGGAAAG AGAGGTGTCC ACAGTGAGGT CCAGTTGACG TGGAGCCAAG ATAGCTAACT TAAGGGGCCG GTATCGACAG

                                                          gsuI/bpmI
                                                         scrFI
                                                         mvaI
                                                         ecoRII                                                mnlI
                                                         dsaV                                                  ecoNI
              mnlI                         nspBII        bstNI                         ddeI                      sau96I
            haeIII/palI                fnu4HI     hgiAI/aspHI                         mnlI                       nlaIV
           sau96I                   fnu4HI aciI   bsp1286                             eco81I                     avaII
           asuI                     bbvI  fnu4HI  bsiHKAI    aluI                     bsu36I/mstII/sauI          asuI  hphI
        nlaIII                 bspMI   bbvI       bmyI apyI[dcm+]                   bslI                   bsrI   bslI
1601 TGGCATGGGC CTCTCCACCG TGCCTGACCT GCTGCTGCCG CTGGTGCTCC TGGAGCTGTT GGTGGGAATA TACCCCTCAG GGGTTATTGG ACTGGTCCCT
     ACCGTACCCG GAGAGGTGGC ACGGACTGGA CGACGACGGC GACCACGAGG ACCTCGACAA CCACCCTTAT ATGGGGAGTC CCCAATAACC TGACCAGGGA

        rmaI
        maeI
       styI
       bsaJI
       blnI            mboII                  styI                                          rsaI
       avrII           earI/ksp632I           bsaJI                mnlI         taqI        csp6I              nlaIV
1701 CACCTAGGGG ACAGGGAGAA GAGAGATAGT GTGTGTCCCC AAGGAAAATA TATCCACCCT CAAAATAATT CGATTTGCTG TACCAAGTGC CACAAAGGAA
     GTGGATCCCC TGTCCCTCTT CTCTCTATCA CACACAGGGG TTCCTTTTAT ATAGGTGGGA GTTTTATTAA GCTAAACGAC ATGGTTCACG GTGTTTCCTT
```

FIG. 6D

```
                                                                                haeIII/palI
                                                                               haeI
                                                          scrFI         scrFI                     nlaIII
                                                          mvaI               mvaI                          sau3AI
                                                          ecoRII        ecoRII                 mboI/ndeII[dam-]
                                                          dsaV   tfiI   dsaV    pleI           dpnI[dam+]
                                                          bstNI     nlaIII   bstNI    ddeI                dpnII[dam-]
          accI  nlaIII              mnlI                  apyI[dcm+]  hinfI  apyI[dcm+]  hinfI    maeIII   alwI[dam-]
1101 CAAGTAAAGT AGACATGGTT TGGATAGTCG GAGGCAGTTC TGTTTACCAG GAAGCCATGA ATCAACCAGG CCACCTTAGA CTCTTTGTGA CAAGGATCAT
     GTTCATTTCA TCTGTACCAA ACCTATCAGC CTCCGTCAAG ACAAATGGTC CTTCGGTACT TAGTTGGTCC GGTGGAATCT GAGAAACACT GTTCCTAGTA

                                                                                         mnlI
                                                                                    hinlI/acyI        scrFI
                                                                                    ahaII/bsaHI       mvaI
                                                                               scrFI        ecoRII
                                                                               mvaI    ecoNI         dsaV
                                                                               ecoRII      sau96I
                                                                               dsaV        avaII
                    maeII                                                      bstNI   bslI     asuI   mnlI
                  aflIII                                                       apyI[dcm+]     mnlI  bstNI
        apoI    maeIII                                       mnlI            bsaJI hgaI     ddeI  apyI[dcm+]
1201 GCAGGAATTT GAAAGTGACA CGTTTTTCCC AGAAATTGAT TTGGGGAAAT ATAAACCTCT CCCAGAATAC CCAGGCGTCC TCTCTGAGGT CCAGGAGGAA
     CGTCCTTAAA CTTTCACTGT GCAAAAAGGG TCTTTAACTA AACCCCTTTA TATTTGGAGA GGGTCTTATG GGTCCGCAGG AGAGACTCCA GGTCCTCCTT

                                                                                            ppu10I
                                                       sfaNI                                nsiI/avaIII
        sfaNI                  accI    mboII       mboII                     mnlI      aluI
1301 AAAGGCATCA AGTATAAGTT TGAAGTCTAC GAGAAGAAAG ACTAACAGGA AGATGCTTTC AAGTTCTCTG CTCCCCTCCT AAAGCTATGC ATTTTTATAA
     TTTCCGTAGT TCATATTCAA ACTTCAGATG CTCTTCTTTC TGATTGTCCT TCTACGAAAG TTCAAGAGAC GAGGGGAGGA TTTCGATACG TAAAAATATT

                                                   fnu4HI
       nlaIII                                      aciI
      styI                                        thaI
      ncoI                                        fnuDII/mvnI tru9I
      dsaI                      styI              bstUI     mseI                                   maeIII
      bsaJI                     bsaJI             bsh1236I aseI/asnI/vspI                         hphI
1401 GACCATGGGA CTTTTGCTGG CTTTAGACCC CCTTGGCTTC GTTAGAACGC GGCTACAATT AATACATAAC CTTATGTATC ATACACATAG ATTTAGGTGA
     CTGGTACCCT GAAAACGACC GAAATCTGGG GGAACCGAAG CAATCTTGCG CCGATGTTAA TTATGTATTG GAATACATAG TATGTGTATC TAAATCCACT
```

FIG. 6C

```
              tfiI
          aciI
         thaI hinfI
         fnuDII/mvnI                                                                             fnu4HI
         bstUI                                           aciI                                    bbvI
         bsh1236I                                     rsaI                                       nspBII          taqI
                                                      csp6I   scfI            mnlI               aciI         nlaIII
 701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG CTGTAAGTAC CGCCTATAGA GCGATAAGAG GATTTTATCC CCGCTGCCAT CATGGTTCGA CCATTGAACT
     AACCTTGCGC CTAAGGGGCA CGGTTCTCAC GACATTCATG GCGGATATCT CGCTATTCTC CTAAAATAGG GGCGACGGTA GTACCAAGCT GGTAACTTGA

                                                                                      thaI
                                                                                      fnuDII/mvnI
                                                                                      bstUI
                                                                                      bsh1236I
                                                                                    mluI
                                                                        bsrBI       aflIII       rsaI
                   pflMI                        bsmAI                   aciI      xmnI           csp6I
     sfaNI         bslI                         bsaI                 mnlI    ddeI asp700        scaI
 801 GCATCGTCGC CGTGTCCCAA AATATGGGGA TTGGCAAGAA CGGAGACCTA CCCTGCCCTC CGCTCAGGAA CGCGTTCAAG TACTTCCAAA GAATGACCAC
     CGTAGCAGCG GCACAGGGTT TTATACCCCT AACCGTTCTT GCCTCTGGAT GGGACGGGAG GCGAGTCCTT GCGCAAGTTC ATGAAGGTTT CTTACTGGTG

                                                                 scrFI
                                                                 mvaI
                                                                 ecoRII
        eco57I                                                   dsaV
       mboII                 tfiI                                bstNI                     tfiI         tru9I           tru9I
      earI/ksp632I           hinfI                               apyI(dcm+)            mboII  taqI      mseI            mseI
     mnlI                  alwNI       hphI                    sexAI            ddeI     hinfI        ahaIII/draI     aseI/asnI/vspI
 901 AACCTCTTCA GTGGAAGGTA AACAGAATCT GGTGATTATG GGTAGGAAAA CCTGGTTCTC CATTCCTGAG AAGAATCGAC CTTTAAAGGA CAGAATTAAT
     TTGGAGAAGT CACCTTCCAT TTGTCTTAGA CCACTAATAC CCATCCTTTT GGACCAAGAG GTAAGGACTC TTCTTAGCTG GAAATTTCCT GTCTTAATTA

                                        aluI
                                       sstI
                                       sacI
                                       hgiJII
                                       hgiAI/aspHI
                                       ecl136II
                                       bsp1286
                                       bsiHKAI
                                       bmyI                                          tru9I                mspI
                                       banII                               sfaNI     mseI                 hpaII
       ddeI                     bslI  mnlI             bstXI         fokI          aflII/bfrI           bsaWI
1001 ATAGTTCTCA GTAGAGAACT CAAAGAACCA CCACGAGGAG CTCATTTTCT TGCCAAAAGT TTGGATGATG CCTTAAGACT TATTGAACAA CCGGAATTGG
     TATCAAGAGT CATCTCTTGA GTTTCTTGGT GGTGCTCCTC GAGTAAAAGA ACGGTTTTCA AACCTACTAC GGAATTCTGA ATAACTTGTT GGCCTTAACC
```

FIG. 6B

```
                                                                      maeII
                                                                      hinlI/acyI                nlaIV
        rsaI                 pleI                                     ahaII/bsaHI               hgiCI
        csp6I           aciI hinfI                  bsmAI             aatII                     banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                 aluI
                                                                                                sstI
                                                                                                sacI
                                                                                                hgiJII
                                                                                                hgiAI/aspHI
                                                                                                ecl136II
                                                                                                bsp1286
                                                                                                bsiHKAI
                                                                  rsaI                          bmyI
                          maeIII    aciI       hgaI       aciI    csp6I     mnlI                banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                         haeIII/palI
                                                                                        mcrI
                                                                                        eagI/xmaIII/eclXI
                                                                                        eaeI
                                                                                        cfrI
                                                                                       fnu4HI
                                                                                       aciI
                                                                                      thaI
                        esp3I                                                         fnuDII/mvnI
                   scrFI                                      sau96I              sacII/sstII
                   mvaI bsmAI                                      avaII               nspBII
                   ecoRII                                     asuI                kspI  scrFI
                   dsaV                                      nlaIV                dsaI  nciI
                   bstNI  hinlI/acyI                            scrFI              bglI bslI  mspI
                   apyI[dcm+]                                   nciI          sau3AI mnlI bstUI
               sau3AI gsuI/bpmI                                 mspI          mboI/ndeII[dam-] hpaII
               mboI/ndeII[dam-]                          mboII  hpaII         dpnI[dam+] bsaJI dsaV
               dpnI[dam+]  hgaI  fokI                    bpuAI  dsaV          dpnII[dam-] bsh1236I
               dpnII[dam-] ahaII/bsaHI            mnlI   bbsI   cauII         alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 6A

```
       aluI
      sstI
      sacI
      hgiJII
      hgiAI/aspHI
      ecl136II
      bsp1286
      bsiHKAI
      bmyI                     rmaI    tru9I
      banII                    maeI    mseI                                                 thaI
     taqI                     speI    aseI/asnI/vspI                                        fnuDII/mvnI
                                                                                            bstUI
                                                                                            bsh1236I
                                                                         bslI              aciI maeIII
  1 TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC
    AAGCTCGAGC GGGCTGTAAC TAATAACTGA TCAATAATTA TCATTAGTTA ATGCCCCAGT AATCAAGTAT CGGGTATATA CCTCAAGGCG CAATGTATTG

                  scrFI
                  mvaI
                  ecoRII
                  dsaV
               aciI
             bglI bstNI                                 maeII
            sau96I                                     hinlI/acyI
            haeIII/palI    aciI                        ahaII/bsaHI
            asuI  apyI[dcm+]              aciI          aatII        maeII          maeIII
101 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA
    AATGCCATTT ACCGGGCGGA CCGACTGGCG GGTTGCTGGG GGCGGGTAAC TGCAGTTATT ACTGCATACA AGGGTATCAT TGCGGTTATC CCTGAAAGGT

      maeII                                                                                 maeII
     hinlI/acyI                                                                            hinlI/acyI
     ahaII/bsaHI                             rsaI                    rsaI                  ahaII/bsaHI
     aatII                         bglI      csp6I          ndeI     csp6I                 aatII
201 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT
    AACTGCAGTT ACCCACCTCA TAAATGCCAT TTGACGGGTG AACCGTCATG TAGTTCACAT AGTATACGGT TCATGCGGGG GATAACTGCA GTTACTGCCA

            scrFI
            mvaI
            ecoRII
        aciI
      bglI  dsaV                                                                           nlaIII
     sau96I bstNI                                                                      styI
     haeIII/palI         rsaI                                         maeII              ncoI
                         csp6I                                 rsaI   snaBI              dsaI hphI  aciI
     asuI   apyI[dcm+]  bsrI  nlaIII                           csp6I  bsaAI              bsaJI   sfaNI
301 AAATGGCCCG CCTGGCATTA TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
    TTTACCGGGC GGACCGTAAT ACGGGTCATG TACTGGAATA CCCTGAAAGG ATGAACCGTC ATGTAGATGC ATAATCAGTA GCGATAATGG TACCACTACG
```

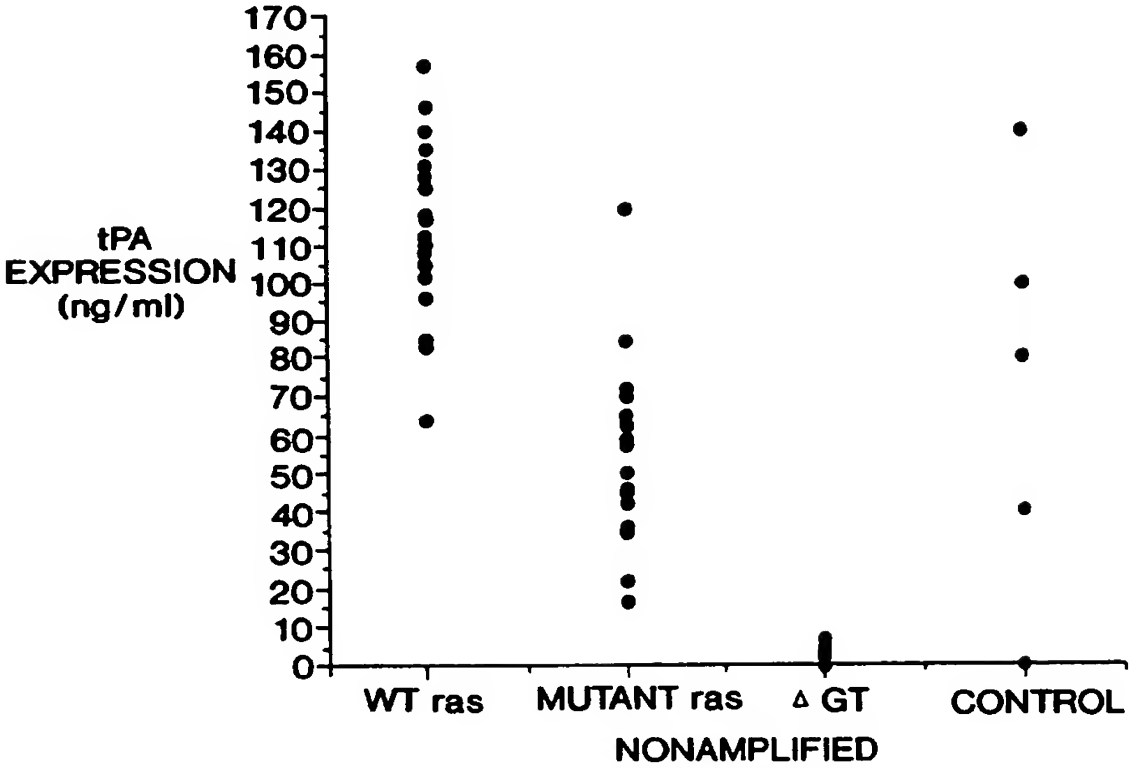

tPA
EXPRESSION
(ng/ml)
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
WT ras
MUTANT ras
Δ GT
CONTROL
NONAMPLIFIED

FIG. 5B

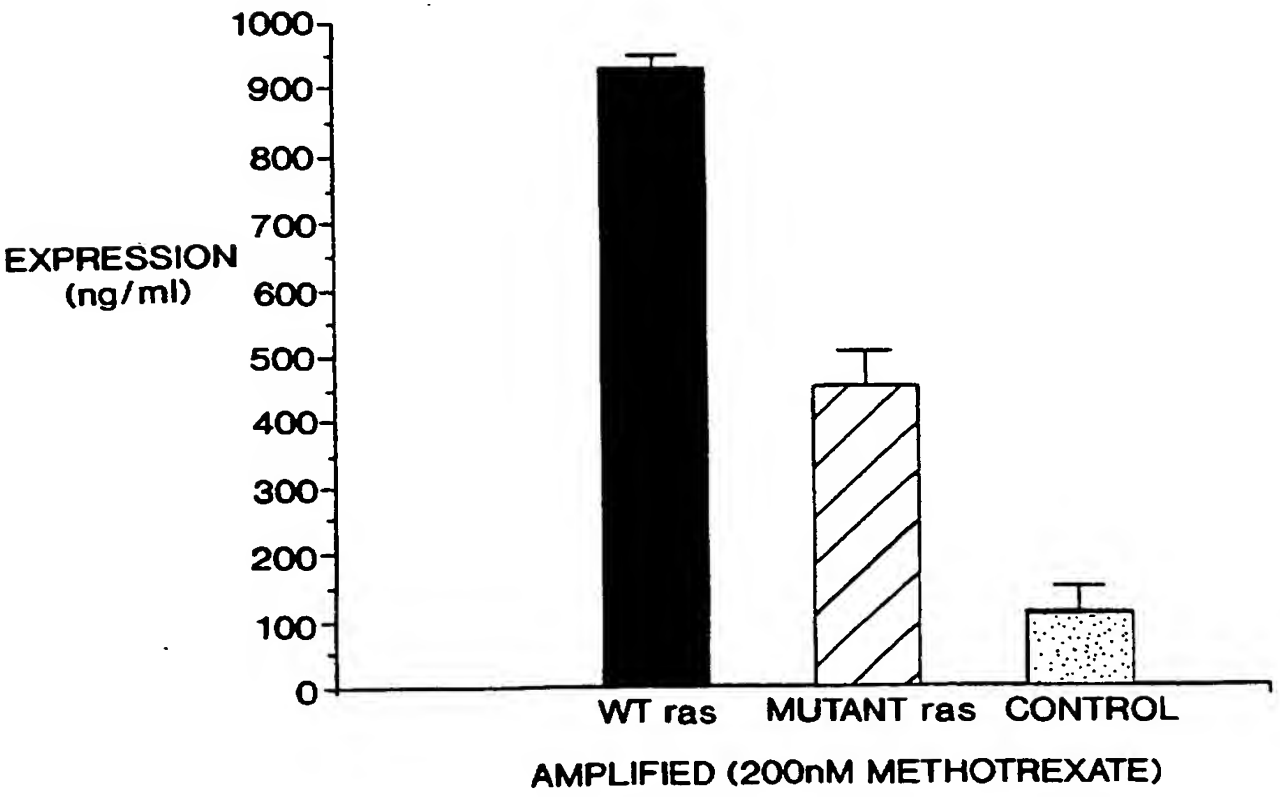

EXPRESSION
(ng/ml)
1000
900
800
700
600
500
400
300
200
100
0
WT ras
MUTANT ras
CONTROL
AMPLIFIED (200nM METHOTREXATE)

FIG. 5C

FIG. 10B

```
                                                                        maeII
                                                                        hinlI/acyI                nlaIV
         rsaI                            pleI                           ahaII/bsaHI               hgiCI
         csp6I                   aciI    hinfI               bsmAI      aatII                     banI
401 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
    CCAAAACCGT CATGTAGTTA CCCGCACCTA TCGCCAAACT GAGTGCCCCT AAAGGTTCAG AGGTGGGGTA ACTGCAGTTA CCCTCAAACA AAACCGTGGT

                                                                                                  aluI
                                                                                                 sstI
                                                                                                 sacI
                                                                                                 hgiJII
                                                                                                 hgiAI/aspHI
                                                                                                 ecl136II
                                                                                                 bsp1286
                                                                                                 bsiHKAI
                                                                             rsaI                bmyI
                             maeIII     aciI       hgaI       aciI           csp6I      mnlI     banII
501 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
    TTTAGTTGCC CTGAAAGGTT TTACAGCATT GTTGAGGCGG GGTAACTGCG TTTACCCGCC ATCCGCACAT GCCACCCTCC AGATATATTC GTCTCGAGCA

                                                                                          haeIII/palI
                                                                                         mcrI
                                                                                         eagI/xmaIII/eclXI
                                                                                         eaeI
                                                                                         cfrI
                                                                                        fnu4HI
                                                                                        aciI
                                                                                       thaI
                       esp3I                                                           fnuDII/mvnI
                   scrFI                                         sau96I              sacII/sstII
                   mvaI bsmAI                                         avaII              nspBII
                   ecoRII                                        asuI                kspI  scrFI
                   dsaV                                         nlaIV                dsaI  nciI
                   bstNI  hinlI/acyI                               scrFI              bglI bslI  mspI
                   apyI[dcm+]                                      nciI          sau3AI mnlI bstUI
               sau3AI gsuI/bpmI                                    mspI          mboI/ndeII[dam-] hpaII
               mboI/ndeII[dam-]                              mboII hpaII         dpnI[dam+] bsaJI dsaV
               dpnI[dam+]  hgaI  fokI                        bpuAI dsaV          dpnII[dam-] bsh1236I
               dpnII[dam-] ahaII/bsaHI              mnlI     bbsI  cauII         alwI[dam-] aciI  cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCGG GAACGGTGCA
    AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAAACTGGA GGTATCTTCT GTGGCCCTGG CTAGGTCGGA GGCGCCGGCC CTTGCCACGT
```

FIG. 10C

```
          tfiI                                                                                   fnu4HI
        aciI                                                                                     aciI
       thaI hinfI                                                               bstXI           thaI
       fnuDII/mvnI                           aciI                            sau96I   styI     fnuDII/mvnI tru9I
       bstUI                        maeII rsaI            pleI scfI haeIII/palI        bstUI       mseI
       bsh1236I                 maeIII    csp6I    scfI hinfI      asuI       bsaJI    bsh1236I   aseI/asnI/vspI
701 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA GTCTATAGGC CCACCCCCTT GGCTTCGTTA GAACGCGGCT ACAATTAATA
    AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCATG GCGGATATCT CAGATATCCG GGTGGGGGAA CCGAAGCAAT CTTGCGCCGA TGTTAATTAT

                                                                                                    sau96I
                                                                                                    avaII
                                                                                                    asuI
                                                                                                 scrFI
                                                                                                 mvaI
                                                                                                 ecoRII
                                                                                                 dsaV
                                                                                                 bstNI
                                     maeIII                                                      apyI[dcm+]
                                    hphI   scfI         fokI                               bslI bsaJI
801 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC
    GTATTGGAAT ACATAGTATG TGTATGCTAA ATCCACTGTG ATATCTTATT GTAGGTGAAA CGGAAAGAGA GGTGTCCACA GGTGAGGGTC CAGGTTGACG

                                                      hinlI/acyI
                                                      ahaII/bsaHI
                                                      aatII
                                                   thaI                      taqI[dam-]
                                                   fnuDII/mvnI             sau3AI               bsmAI
                  aluI  taqI                       bstUI                   mboI/ndeII[dam-]    esp3I
     mnlI        hindIII                         aciI maeII                dpnI[dam+]      tth111I/aspI
     bsaJI   ddeI     claI/bsp106            hphI bsh1236I     taqI        dpnII[dam-] taqI   hgaI          sfaNI
901 ACCTCGGTTC TAAGCTTATC GATATGAAAA AGCCTGAACT CACCGCGACG TCTGTCGAGA AGTTTCTGAT CGAAAAGTTC GACAGCGTCT CCGACCTGAT
    TGGAGCCAAG ATTCGAATAG CTATACTTTT TCGGACTTGA GTGGCGCTGC AGACAGCTCT TCAAAGACTA GCTTTTCAAG CTGTCGCAGA GGCTGGACTA

                                                                                              hinPI
      aluI                tfiI                                                            fnu4HI
    fnu4HI                hinfI                taqI                                       bbvI
    bbvI        mnlI  mboII                aluI           mnlI             aciI          aluI hhaI/cfoI
1001 GCAGCTCTCG GAGGGCGAAG AATCTCGTGC TTTCAGCTTC GATGTAGGAG GGCGTGGATA TGTCCTGCGG GTAAATAGCT GCGCCGATGG TTTCTACAAA
     CGTCGAGAGC CTCCCGCTTC TTAGAGCACG AAAGTCGAAG CTACATCCTC CCGCACCTAT ACAGGACGCC CATTTATCGA CGCGGCTACC AAAGATGTTT
```

FIG. 10D

```
                                    hinPI
                                    hhaI/cfoI    mspI
                                   thaI          hpaII
                                  aciI          mroI
                               haeIII/palI      bspMII
                              mcrI fnuDII/mvnI  bspEI
     sau3AI                   eagI/xmaIII/eclXI bsaWI
     mboI/ndeII(dam-)         eaeI bstUI    tfiI
     dpnI(dam+)               cfrI bsh1236I hinfI                ecoRI
     dpnII(dam-)          sfaNI fnu4HI  bslI   accIII            apoI                        sfaNI  aciI
1101 GATCGTTATG TTTATCGGCA CTTTGCATCG GCCGCGCTCC CGATTCCGGA AGTGCTTGAC ATTGGGGAAT TCAGCGAGAG CCTGACCTAT TGCATCTCCC
     CTAGCAATAC AAATAGCCGT GAAACGTAGC CGGCGCGAGG GCTAAGGCCT TCACGAACTG TAACCCCTTA AGTCGCTCTC GGACTGGATA ACGTAGAGGG

                                                                                                      mcrI
                                                                                                      eagI/xmaIII/eclXI
                                                                                                      eaeI
                                                                                  styI               fnu4HI
                                                                         thaI     ncoI       sau3AI  aciI
       hgiAI/aspHI                                                       fnuDII/mvnI         mboI/ndeII(dam-)
       bsp1286                                                   fnu4HI  bstUI    dsaI       dpnI(dam+)   sau3AI
       bsiHKAI                                                   bbvI  mcrI     haeIII/palI  dpnII(dam-)  mboI/ndeII(dam-)
       bmyI                                                    scfI   mspI bsh1236I bsaJI sfaNI   fnu4HI  dpnI(dam+)
       apaLI/snoI                                              
       alw44I/snoI    maeII                             nspBII pstI   hpaII    mnlI nlaIII  pvuI/bspCI haeIII/palI
      bslI draIII   maeIII      bspMI                   aciI   bsgI  cfr10I aciI haeI   fokI  mcrI bbvI cfrI dpnII(dam-)
1201 GCCGTGCACA GGGTGTCACG TTGCAACACC TGCCTGAAAC CGAACTGCCC GCTGTTCTGC AGCCGGTCGC GGAGGCCATG GATGCGATCG CTGCGGCCGA
     CGGCACGTGT CCCACAGTGC AACGTTGTGG ACGGACTTTG GCTTGACGGG CGACAAGACG TCGGCCAGCG CCTCCGGTAC CTACGCTAGC GACGCCGGCT

                                   sau96I                                                     thaI      sau3AI
                                   avaII                                                      fnuDII/mvnI     nlaIII
                                   asuI                                                       bstUI     mboI/ndeII(dam-)
                          sau96I  rsrII/cspI                                                  bsh1236I  dpnI(dam+)
                aciI      haeIII/palI  aciI    tfiI                                          hinPI      dpnII(dam-)
      ddeI     bsrBI      asuI    cpoI         hinfI                nlaIII            ndeI hhaI/cfoI    alwI(dam-)
1301 TCTTAGCCAG ACGAGCGGGT TCGGCCCATT CGGACCGCAA GGAATCGGTC AATACACTAC ATGGCGTGAT TTCATATGCG CGATTGCTGA TCCCCATGTG
     AGAATCGGTC TGCTCGCCCA AGCCGGGTAA GCCTGGCGTT CCTTAGCCAG TTATGTGATG TACCGCACTA AAGTATACGC GCTAACGACT AGGGGTACAC

                                                 hinPI                                                             draIII
                                                 hhaI/cfoI                                                       nlaIV
                                                thaI                                        bsaJI                hgiCI
                                                fnuDII/mvnI                               haeIII/palI            banI
                                tth111I/aspI    bstUI                          sfaNI     sau96I                 mspI
        bsrI                  drdI           hgaI      bsh1236I    taqI    aluI          asuI mnlI      bslI    hpaII
1401 TATCACTGGC AAACTGTGAT GGACGACACC GTCAGTGCGT CCGTCGCGCA GGCTCTCGAT GAGCTGATGC TTTGGGCCGA GGACTGCCCC GAAGTCCGGC
     ATAGTGACCG TTTGACACTA CCTGCTGTGG CAGTCACGCA GGCAGCGCGT CCGAGAGCTA CTCGACTACG AAACCCGGCT CCTGACGGGG CTTCAGGCCG
```

FIG. 10E

```
          aciI
         thaI
         fnuDII/mvnI
   hgiAI/aspHI
   bsp1286
   bsiHKAI
   bmyI  bstUI                                   aciI
   apaLI/snoI                                   fnu4HI
   alw44I/snoI                                 haeIII/palI
                                              eaeI         aciI        gsuI/bpmI               tfiI bslI
  mnlI   bsh1236I   nlaIV                     cfrI       nspBII      bsrI      mnlI           hinfI
1501 ACCTCGTGCA CGCGGATTTC GGCTCCAACA ATGTCCTGAC GGACAATGGC CGCATAACAG CGGTCATTGA CTGGAGCGAG GCGATGTTCG GGGATTCCCA
     TGGAGCACGT GCGCCTAAAG CCGAGGTTGT TACAGGACTG CCTGTTACCG GCGTATTGTC GCCAGTAACT GACCTCGCTC CGCTACAAGC CCCTAAGGGT

                                                                                                   fnu4HI
                                                                                                  thaI
                                                                                                  fnuDII/mvnI
                                                                                                  bstUI
                                                                                                  bsh1236I
                                                                                                 sacII/sstII
                                                                                                 nspBII
                                                                                                 kspI
                                                                                 mspI            dsaI
                                                                                 hpaII           bsaJI
                                                                                mroI             aciI
                                                                                bspMII          fnu4HI
                                                                                bspEI         sau3AI aciI
                                    dsaI                                        bsaWI         mboI/ndeII[dam-]
                                 haeIII/palI                     rsaI     aciI   fokI         dpnI[dam+]
                         mboII  mnlI bsaJI             fnu4HI    csp6I  bsrBI   sfaNI   aluI  dpnII[dam-]
    mnlI             mboII   gsuI/bpmI                bbvI   maeII   taqI    mnlI  accIII     alwI[dam-]
1601 ATACGAGGTC GCCAACATCT TCTTCTGGAG GCCGTGGTTG GCTTGTATGG AGCAGCAGAC GTACTTCGAG CGGAGGCATC CGGAGCTTGC AGGATCGCCG
     TATGCTCCAG CGGTTGTAGA AGAAGACCTC CGGCACCAAC CGAACATACC TCGTCGTCTG CATGAAGCTC GCCTCCGTAG GCCTCGAACG TCCTAGCGGC

      scrFI
      nciI
      mspI
      hpaII                                                                     aluI
      dsaV                                                                     fnu4HI                    hgaI
      cauII                                                                    bbvI      hinPI    taqI   drdI
   nlaIV              aciI                      aluI   nincII/hindII taqI  sfaNI       hhaI/cfoI  sfaNI
1701 CGGCTCCGGG CGTATATGCT CCGCATTGGT CTTGACCAAC TCTATCAGAG CTTGGTTGAC GGCAATTTCG ATGATGCAGC TTGGGCGCAG GGTCGATGCG
     GCCGAGGCCC GCATATACGA GGCGTAACCA GAACTGGTTG AGATAGTCTC GAACCAACTG CCGTTAAAGC TACTACGTCG AACCCGCGTC CCAGCTACGC
```

FIG. 10F

```
          nlaIV                                                          haeIII/palI
         mspI                                                           mcrI
         hpaII  scrFI                                                   eagI/xmaIII/eclXI
         bslI   nciI                                                    eaeI
        mroI    mspI                                                    cfrI
        bspMII  hpaII                                                  fnu4HI
        bspEI[dam-]                                                    aciI
        bsaWI   dsaV                                                  thaI
        accIII[dam-]                                                  fnuDII/mvnI
       sau3AI   cauII                                                 bstUI
       mboI/ndeII[dam-]                                               bsh1236I  sau96I             rsaI
       dpnI[dam+]                                                    hinPI     avaII              csp6I
       dpnII[dam-]             rsaI                                  hhaI/cfoI asuI               scaI
       alwI[dam-]              csp6I        aciI
1801 ACGCAATCGT CCGATCCGGA GCCGGGACTG TCGGGCGTAC ACAAATCGCC CGCAGAAGCG CGGCCGTCTG GACCGATGGC TGTGTAGAAG TACTCGCCGA
     TGCGTTAGCA GGCTAGGCCT CGGCCCTGAC AGCCCGCATG TGTTTAGCGG GCGTCTTCGC GCCGGCAGAC CTGGCTACCG ACACATCTTC ATGAGCGGCT

                                                                      scrFI
                                                                      nciI
                                                                      mspI
                                                                      hpaII
                                                                      dsaV
                                                                     xmaI/pspAI
                                                                     smaI
                                                                     scrFI
                                                                     nciI
                                                                     dsaV
                                                                     cauII
                                                                     bsaJI
                                                                     avaI
                                                                    bsaJI
                                                                  sau3AI                              sau96I
                                                                  mboI/ndeII[dam-]          aciI      haeIII/palI
                                                                  dpnI[dam+]               fnu4HI     asuI
                                                                  dpnII[dam-]              bglI  nlaIII
                                                                  alwI[dam-]              sfiI  styI
                                                                 nlaIV cauII             eaeI   ncoI
      hinlI/acyI                                        mcrI     bstYI/xhoII             cfrI   dsaI
      hgaI                   mnlI                      bslI      bamHI bsaJI ecoRI     taqI haeIII/palI
      ahaII/bsaHI          bsaJI                     sfaNI       alwI[dam-]  apoI     claI/bsp106 bsaJI
1901 TAGTGGAAAC CGACGCCCCA GCACTCGTCC GAGGGCAAAG GAATAGAGTA GATGCCGACC GAAGGATCCC CGGGGAATTC AATCGATGGC CGCCATGGCC
     ATCACCTTTG GCTGCGGGGT CGTGAGCAGG CTCCCGTTTC CTTATCTCAT CTACGGCTGG CTTCCTAGGG GCCCCTTAAG TTAGCTACCG GCGGTACCGG
```

FIG. 10G

```
                                                                                                   rmaI
                     aluI
                   fnu4HI
                   bbvI          maeIII              sfaNI   apoI                            bsmI maeI
2001 CAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG
     GTTGAACAAA TAACGTCGAA TATTACCAAT GTTTATTTCG TTATCGTAGT GTTTAAAGTG TTTATTTCGT AAAAAAAGTG ACGTAAGATC AACACCAAAC

                                            sau3AI
                                            mboI/ndeII[dam-]
                                            dpnI[dam+]
                                            dpnII[dam-]
                                           pvuI/bspCI
                                           mcrI
                                          taqI[dam-]  tru9I                  haeIII/palI
                                         claI/bsp106[dam-]                  haeI
                                        sau3AI        mseI       fnu4HI styI
                                        mboI/ndeII[dam-]         bbvI   ncoI
                                        dpnI[dam+] xmnI        hinPI    dsaI
                                        dpnII[dam-]  aseI/asnI/vspI     bsaJI
                               nlaIII  alwI[dam-]  asp700      hhaI/cfoI nlaIII          mnlI      mnlI
2101 TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCGATC GGGAATTAAT TCGGCGCAGC ACCATGGCCT GAAATAACCT CTGAAAGAGG AACTTGGTTA
     AGGTTTGAGT AGTTACATAG AATAGTACAG ACCTAGCTAG CCCTTAATTA AGCCGCGTCG TGGTACCGGA CTTTATTGGA GACTTTCTCC TTGAACCAAT

      rsaI                                                                     nlaIV
      csp6I                                                                   scrFI                         sfaNI
     nlaIV                                                                    mvaI                              ppu10I
     kpnI                                                                     ecoRII                      nsiI/avaIII
     hgiCI                                                                    dsaV                       nlaIII
     banI                       aluI                                          bstNI                          sphI
     asp718     mnlI           pvuII                                          apyI[dcm+]                     nspI
     acc65I ddeI  aciI         nspBII                                        bsaJI                           nspHI
2201 GGTACCTTCT GAGGCGGAAA GAACCAGCTG TGGAATGTGT GTCAGTTAGG GTGTGGAAAG TCCCCAGGCT CCCCAGCAGG CAGAAGTATG CAAAGCATGC
     CCATGGAAGA CTCCGCCTTT CTTGGTCGAC ACCTTACACA CAGTCAATCC CACACCTTTC AGGGGTCCGA GGGGTCGTCC GTCTTCATAC GTTTCGTACG

                                              nlaIV
                        scrFI        scrFI
                        mvaI               mvaI                                   ppu10I
                        ecoRII       ecoRII                             nsiI/avaIII
                        dsaV         dsaV                              nlaIII
                        bstNI              bstNI                                 sphI
                        apyI[dcm+]         apyI[dcm+]                            nspI sfaNI
                       sexAI              bsaJI                                  nspHI                          aciI
2301 ATCTCAATTA GTCAGCAACC AGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT GCATCTCAAT TAGTCAGCAA CCATAGTCCC
     TAGAGTTAAT CAGTCGTTGG TCCACACCTT TCAGGGGTCC GAGGGGTCGT CCGTCTTCAT ACGTTTCGTA CGTAGAGTTA ATCAGTCGTT GGTATCAGGG
```

FIG. 10H

```
                                                                                                    fnu4HI
                                                                                                    bglI
                                                                 nlaIII                            sfiI
                                                                styI                               haeIII/palI
                                                                ncoI                              mnlI
                     aciI                bsrI              bslI dsaI                           haeIII/palI
            aciI fokI                aciI         aciI     aciI bsaJI                       mnlI bsaJI aciI
2401 GCCCCTAACT CCGCCCATCC CGCCCCTAAC TCCGCCCAGT TCCGCCCATT CTCCGCCCCA TGGCTGACTA ATTTTTTTTA TTTATGCAGA GGCCGAGGCC
     CGGGGATTGA GGCGGGTAGG GCGGGGATTG AGGCGGGTCA AGGCGGGTAA GAGGCGGGGT ACCGACTGAT TAAAAAAAAT AAATACGTCT CCGGCTCCGG

                                                                                          scrFI
                                                                                          nciI
                                                                                          mspI
                                                                                          hpaII
                                                                                          dsaV
                                                          styI                          haeIII/palI
                                                          bsaJI                       mcrI
                                                          blnI                        eagI/xmaIII/eclXI
                                                       haeIII/palI          rmaI      eaeI
         haeIII/palI                                  stuI rmaI             maeI      cfrI
     mnlI      ddeI                     mnlI          haeI maeI            nheI      mspI cauII
     bsaJI mnlI   aluI               mnlI            mnlI avrII          aluI aluI  hpaII
2501 GCCTCGGCCT CTGAGCTATT CCAGAAGTAG TGAGGAGGCT TTTTTGGAGG CCTAGGCTTT TGCAAAAAGC TAGCTTATCC GGCCGGGAAC GGTGCATTGG
     CGGAGCCGGA GACTCGATAA GGTCTTCATC ACTCCTCCGA AAAAACCTCC GGATCCGAAA ACGTTTTTCG ATCGAATAGG CCGGCCCTTG CCACGTAACC

         tfiI
         hinfI                                                                             fnu4HI
       aciI                                                                                aciI
      thaI                                                      bstXI                    thaI
      fnuDII/mvnI                          aciI         scfI  sau96I   styI              fnuDII/mvnI  tru9I
      bstUI                pleI        rsaI        pleI    haeIII/palI                   bstUI        mseI
      bsh1236I             hinfI       csp6I  scfI  hinfI  asuI        bsaJI             bsh1236I   aseI/asnI/vspI
2601 AACGCGGATT CCCCGTGCCA AGAGTCAGGT AAGTACCGCC TATAGAGTCT ATAGGCCCAC CCCCTTGGCT TCGTTAGAAC GCGGCTACAA TTAATACATA
     TTGCGCCTAA GGGGCACGGT TCTCAGTCCA TTCATGGCGG ATATCTCAGA TATCCGGGTG GGGGAACCGA AGCAATCTTG CGCCGATGTT AATTATGTAT
```

FIG. 10I

```
                sau3AI
                mboI/ndeII[dam-]
                dpnI[dam+]                                                    sau96I
                dpnII[dam-]                                                   avaII
                alwI[dam-]                                                    asuI
              taqI[dam-]                                                    scrFI
             claI/bsp106[dam-]                                              mvaI                              rmaI
            sau3AI                                                          ecoRII              thaI    maeI
            mboI/ndeII[dam-]                                                dsaV                fnuDII/mvnI
            dpnI[dam+]                                                      bstNI                     bstUI  nheI
            dpnII[dam-]                                                     apyI[dcm+]        mnlI    bsh1236I  aluI
           alwI[dam-]              fokI                              bslI  bsaJI             bsaJI  nruI   aluI
2701 ACCTTTTGGA TCGATCCTAC TGACACTGAC ATCCACTTTT TCTTTTTCTC CACAGGTGTC CACTCCCAGG TCCAACTGCA CCTCGGTTCG CGAAGCTAGC
     TGGAAAACCT AGCTAGGATG ACTGTGACTG TAGGTGAAAA AGAAAAAGAG GTGTCCACAG GTGAGGGTCC AGGTTGACGT GGAGCCAAGC GCTTCGATCG

                            nlaIII
                           styI
                           pflMI
                           ncoI
          sfaNI     ecoRI  dsaI                                                rsaI              aluI
       fnu4HI taqI  apoI    bslI fokI                          rmaI          gsuI/bpmI            pvuII tth111I/aspI
       bbvI claI/bsp106     bsaJI       nlaIII  fokI         maeI          bsrI   csp6I     ecoRV nspBII   bsrI
2801 TTGGGCTGCA TCGATTGAAT TCCACCATGG GATGGTCATG TATCATCCTT TTTCTAGTAG CAACTGCAAC TGGAGTACAT TCAGATATCC AGCTGACCCA
     AACCCGACGT AGCTAACTTA AGGTGGTACC CTACCAGTAC ATAGTAGGAA AAAGATCATC GTTGACGTTG ACCTCATGTA AGTCTATAGG TCGACTGGGT

          aluI
         sstI
         sacI
         hgiJII
         hgiAI/aspHI
         ecl136II
         bsp1286
         bsiHKAI
         bmyI                          hphI
         banII          mnlI           maeIII    bspMI                taqI                          bsrI
      avaI          aciI               bstEII   hphI        bsrI      hgaI          hphI   aluI  nlaIII
2901 GTCCCCGAGC TCCCTGTCCG CCTCTGTGGG CGATAGGGTC ACCATCACCT GCCGTGCCAG TCAGAGCGTC GATTACGATG GTGATAGCTA CATGAACTGG
     CAGGGGCTCG AGGGACAGGC GGAGACACCC GCTATCCCAG TGGTAGTGGA CGGCACGGTC AGTCTCGCAG CTAATGCTAC CACTATCGAT GTACTTGACC
```

FIG. 10J

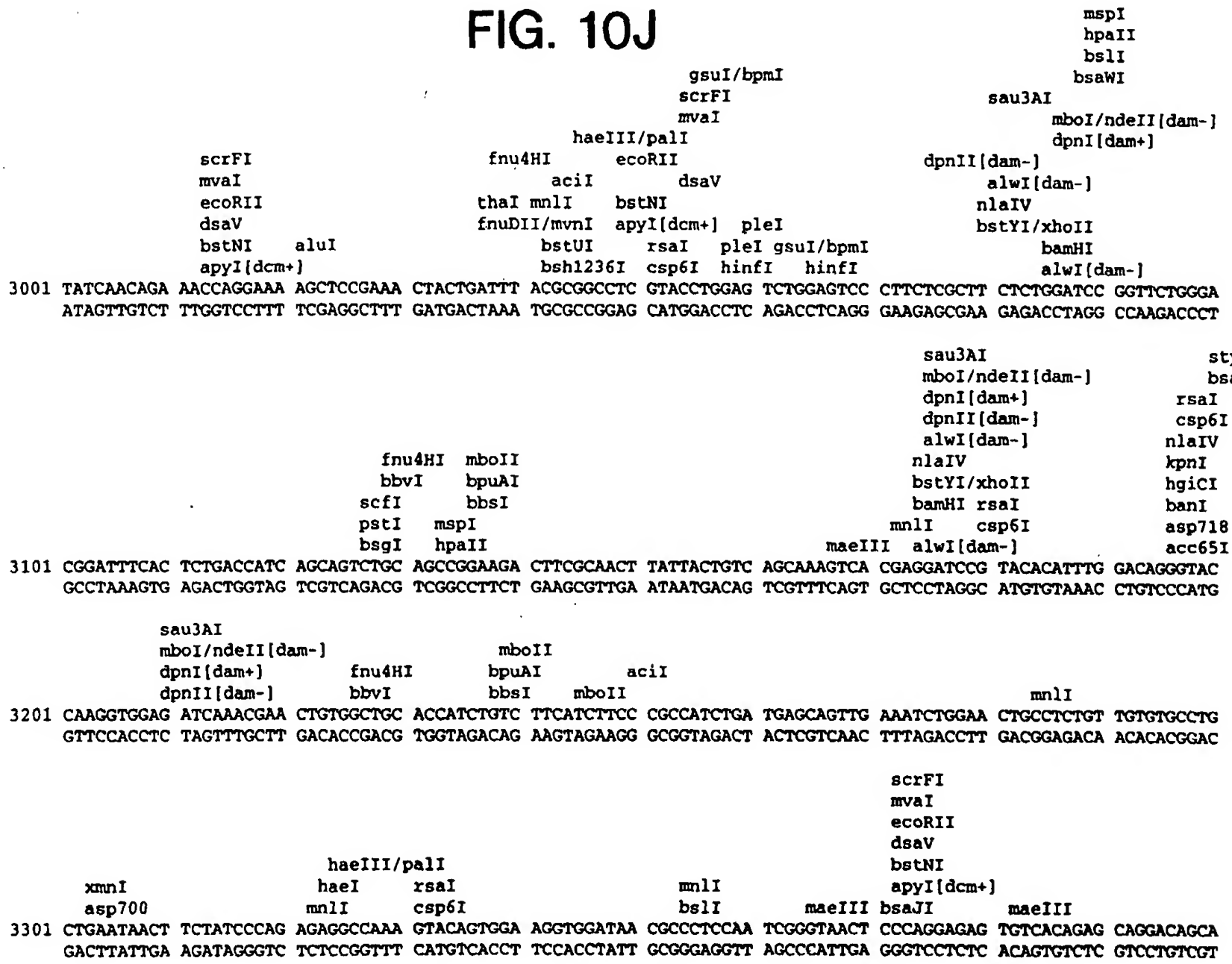

mspI
hpaII
bslI
bsaWI
gsuI/bpmI
scrFI
mvaI
sau3AI
haeIII/palI
mboI/ndeII[dam-]
dpnI[dam+]
scrFI
fnu4HI
ecoRII
dpnII[dam-]
mvaI
aciI
dsaV
alwI[dam-]
ecoRII
thaI mnlI
bstNI
nlaIV
dsaV
fnuDII/mvnI
apyI[dcm+]
pleI
bstYI/xhoII
bstNI
aluI
bstUI
rsaI
pleI gsuI/bpmI
bamHI
apyI[dcm+]
bsh1236I
csp6I
hinfI
hinfI
alwI[dam-]
3001 TATCAACAGA AACCAGGAAA AGCTCCGAAA CTACTGATTT ACGCGGCCTC GTACCTGGAG TCTGGAGTCC CTTCTCGCTT CTCTGGATCC GGTTCTGGGA
ATAGTTGTCT TTGGTCCTTT TCGAGGCTTT GATGACTAAA TGCGCCGGAG CATGGACCTC AGACCTCAGG GAAGAGCGAA GAGACCTAGG CCAAGACCCT
sau3AI
styI
mboI/ndeII[dam-]
bsaJI
dpnI[dam+]
rsaI
dpnII[dam-]
csp6I
alwI[dam-]
nlaIV
fnu4HI
mboII
nlaIV
kpnI
bbvI
bpuAI
bstYI/xhoII
hgiCI
scfI
bbsI
bamHI
rsaI
banI
pstI
mspI
mnlI
csp6I
asp718
bsgI
hpaII
maeIII
alwI[dam-]
acc65I
3101 CGGATTTCAC TCTGACCATC AGCAGTCTGC AGCCGGAAGA CTTCGCAACT TATTACTGTC AGCAAAGTCA CGAGGATCCG TACACATTTG GACAGGGTAC
GCCTAAAGTG AGACTGGTAG TCGTCAGACG TCGGCCTTCT GAAGCGTTGA ATAATGACAG TCGTTTCAGT GCTCCTAGGC ATGTGTAAAC CTGTCCCATG
sau3AI
mboI/ndeII[dam-]
mboII
dpnI[dam+]
fnu4HI
bpuAI
aciI
dpnII[dam-]
bbvI
bbsI
mboII
mnlI
3201 CAAGGTGGAG ATCAAACGAA CTGTGGCTGC ACCATCTGTC TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
GTTCCACCTC TAGTTTGCTT GACACCGACG TGGTAGACAG AAGTAGAAGG GCGGTAGACT ACTCGTCAAC TTTAGACCTT GACGGAGACA ACACACGGAC
scrFI
mvaI
ecoRII
dsaV
bstNI
haeIII/palI
apyI[dcm+]
xmnI
haeI
rsaI
mnlI
asp700
mnlI
csp6I
bslI
maeIII
bsaJI
maeIII
3301 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA
GACTTATTGA AGATAGGGTC TCTCCGGTTT CATGTCACCT TCCACCTATT GCGGGAGGTT AGCCCATTGA GGGTCCTCTC ACAGTGTCTC GTCCTGTCGT

FIG. 10K

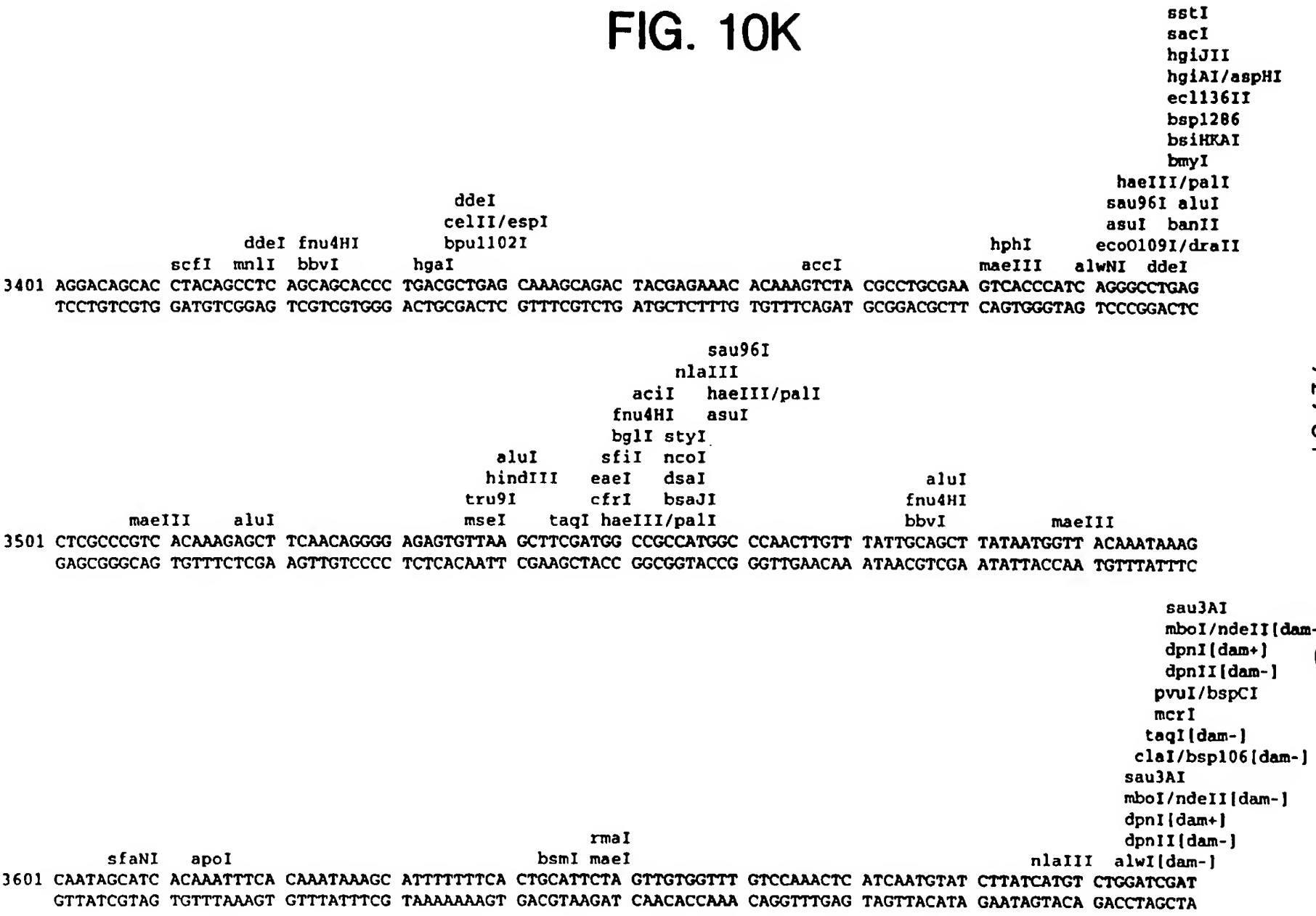


FIG. 10L

```
                                                                        rsaI
                                 haeIII/palI                            csp6I
                                haeI                                   nlaIV
           tru9I      fnu4HI styI                                      kpnI
           mseI       bbvI   ncoI                                      hgiCI
          aseI/asnI/vspI     dsaI                                      banI                       aluI
        xmnI        hinPI    bsaJI                                     asp718     mnlI           pvuII
        asp700      hhaI/cfoI nlaIII          mnlI      mnlI           acc65I  ddeI  aciI        nspBII
3701 CGGGAATTAA TTCGGCGCAG CACCATGGCC TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA AGAACCAGCT GTGGAATGTG
     GCCCTTAATT AAGCCGCGTC GTGGTACCGG ACTTTATTGG AGACTTTCTC CTTGAACCAA TCCATGGAAG ACTCCGCCTT TCTTGGTCGA CACCTTACAC

                                  nlaIV                                                                         nlaIV
                               scrFI                          sfaNI                scrFI        scrFI
                               mvaI                               ppu10I                 mvaI                mvaI
                               ecoRII                       nsiI/avaIII            ecoRII       ecoRII
                               dsaV                        nlaIII                  dsaV         dsaV
                               bstNI                            sphI                     bstNI               bstNI
                               apyI[dcm+]                       nspI                     apyI[dcm+]          apyI[dcm+]
                              bsaJI                             nspHI                   sexAI               bsaJI
3801 TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC CAGGTGTGGA AAGTCCCCAG
     ACAGTCAATC CCACACCTTT CAGGGGTCCG AGGGGTCGTC CGTCTTCATA CGTTTCGTAC GTAGAGTTAA TCAGTCGTTG GTCCACACCT TTCAGGGGTC

                                    ppu10I
                                    nsiI/avaIII
                                   nlaIII
                                  sphI
                                  nspI sfaNI                                                                     bsrI
                                  nspHI                           aciI          aciI fokI aciI         aciI
3901 GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC CCGCCCCTAA CTCCGCCCAG
     CGAGGGGTCG TCCGTCTTCA TACGTTTCGT ACGTAGAGTT AATCAGTCGT TGGTATCAGG GCGGGGATTG AGGCGGGTAG GGCGGGGATT GAGGCGGGTC

                                                                   fnu4HI
                                                                   bglI
                         nlaIII                                   sfiI
                        styI                                      haeIII/palI
                        ncoI                                    mnlI    mnlI      ddeI
                   bslI dsaI                                haeIII/palI bsaJI mnlI   aluI                     mnlI
       aciI        aciI bsaJI                            mnlI bsaJI aciI    haeIII/palI                      mnlI
4001 TTCCGCCCAT TCTCCGCCCC ATGGCTGACT AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT TCCAGAAGTA GTGAGGAGGC
     AAGGCGGGTA AGAGGCGGGG TACCGACTGA TTAAAAAAAA TAAATACGTC TCCGGCTCCG GCGGAGCCGG AGACTCGATA AGGTCTTCAT CACTCCTCCG
```

FIG. 10M

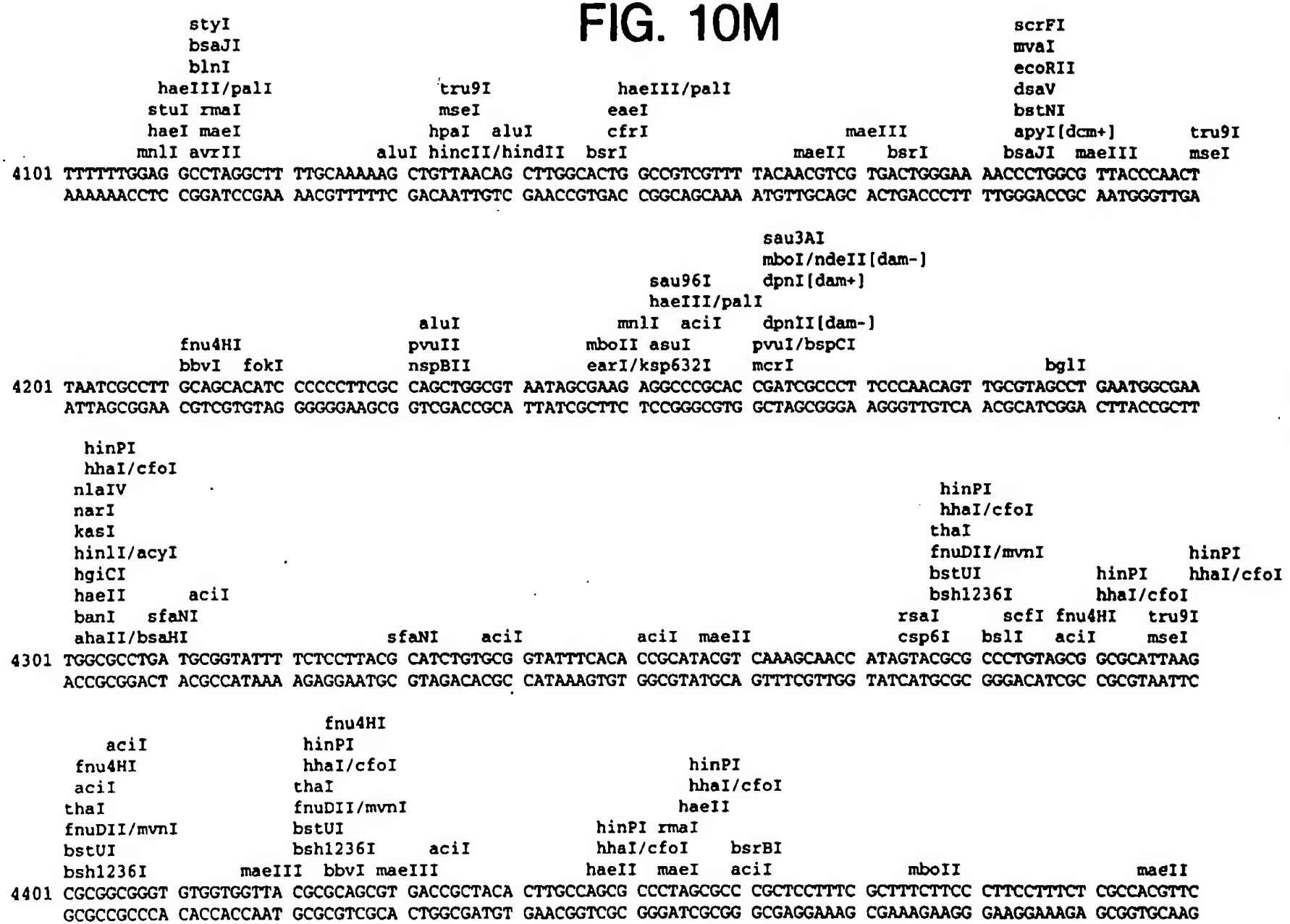


FIG. 10N

```
                                     nlaIV
        mspI                         hgiJII                                                   mnlI
        hpaII                        bsp1286                                             nlaIV
       naeI                          bmyI                                                hgiCI
       cfr10I            aluI        banII        nlaIV                                  banI     taqI                  hphI
4501 GCCGGCTTTC CCCGTCAAGC TCTAAATCGG GGGCTCCCTT TAGGGTTCCG ATTTAGTGCT TTACGGCACC TCGACCCCAA AAAACTTGAT TTGGGTGATG
     CGGCCGAAAG GGGCAGTTCG AGATTTAGCC CCCGAGGGAA ATCCCAAGGC TAAATCACGA AATGCCGTGG AGCTGGGGTT TTTTGAACTA AACCCACTAC

          maeII    haeIII/palI
         draIII   sau96I                                        maeII  pleI           tru9I      pleI
         bsaAI    asuI                                         drdI   hinfI maeII    mseI       hinfI       bsrI
4601 GTTCACGTAG TGGGCCATCG CCCTGATAGA CGGTTTTTCG CCCTTTGACG TTGGAGTCCA CGTTCTTTAA TAGTGGACTC TTGTTCCAAA CTGGAACAAC
     CAAGTGCATC ACCCGGTAGC GGGACTATCT GCCAAAAAGC GGGAAACTGC AACCTCAGGT GCAAGAAATT ATCACCTGAG AACAAGGTTT GACCTTGTTG

                                                                                                        thaI
                                                                                                        fnuDII/mvnI
                                                                                                        bstUI
                                                                         tru9I                          bsh1236I
           bslI                                                          mseI             tru9I       tru9I
          bslI  avaI                                          haeIII/palI          aluI  mseI    apoI mseI    apoI
4701 ACTCAACCCT ATCTCGGGCT ATTCTTTTGA TTTATAAGGG ATTTTGCCGA TTTCGGCCTA TTGGTTAAAA AATGAGCTGA TTTAACAAAA ATTTAACGCG
     TGAGTTGGGA TAGAGCCCGA TAAGAAAACT AAATATTCCC TAAAACGGCT AAAGCCGGAT AACCAATTTT TTACTCGACT AAATTGTTTT TAAATTGCGC

                                          hgiAI/aspHI
                                          bsp1286
                   maeII                  bsiHKAI                                                            tth111I/aspI
                tru9I                     bmyI    ddeI                        aciI                         maeIII
       tru9I    mseI                      apaLI/snoI  rsaI                   fnu4HI    tru9I              maeII
       mseI  sspI  psp1406I               alw44I/snoI csp6I               sfaNI       mseI       aciI      bsaAI bsrI
4801 AATTTTAACA AAATATTAAC GTTTACAATT TTATGGTGCA CTCTCAGTAC AATCTGCTCT GATGCCGCAT AGTTAAGCCA ACTCCGCTAT CGCTACGTGA
     TTAAAATTGT TTTATAATTG CAAATGTTAA AATACCACGT GAGAGTCATG TTAGACGAGA CTACGGCGTA TCAATTCGGT TGAGGCGATA GCGATGCACT

                                                   hinPI
                                                   hhaI/cfoI                  mspI
                                                  thaI                         hpaII
             hinPI                                fnuDII/mvnI                 scrFI                        bsmAI
          fnu4HI                                  bstUI                       nciI     aciI               esp3I
          bbvI                            nspBII  bsh1236I                    dsaV  sfaNI                bslI
      nlaIII  hhaI/cfoI    aciI           aciI  hgaI       drdI               cauII fokI          aluI maeIII
4901 CTGGGTCATG GCTGCGCCCC GACACCCGCC AACACCCGCT GACGCGCCCT GACGGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
     GACCCAGTAC CGACGCGGGG CTGTGGGCGG TTGTGGGCGA CTGCGCGGGA CTGCCCGAAC AGACGAGGGC CGTAGGCGAA TGTCTGTTCG ACACTGGCAG
```

FIG. 100

```
                                                     thaI
                                                     fnuDII/mvnI
                                                     bstUI
                                                     bsh1236I
     scrFI                                          hinPI
     nciI                                           hhaI/cfoI                         mnlI
     mspI       nspI                               thaI                             haeIII/palI
     hpaII fnu4HI                                  fnuDII/mvnI         mboII       sau96I
     dsaV aluI nspHI                               bstUI mnlI          bpuAI       asuI
     cauII bbvI nlaIII      mnlI    hphI       hphI   bsh1236I            bbsI      eco0109I/draII
5001 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GAGGCAGTAT TCTTGAAGAC GAAAGGGCCT CGTGATACGC CTATTTTTAT
     AGGCCCTCGA CGTACACAGT CTCCAAAAGT GGCAGTAGTG GCTTTGCGCG CTCCGTCATA AGAACTTCTG CTTTCCCGGA GCACTATGCG GATAAAAATA

                                                                         nlaIV
                                                                       aciI
                                                                      thaI
                                                                      fnuDII/mvnI
                                            maeII                     bstUI
            nlaIII                         hinlI/acyI                 bsh1236I
       tru9I rcaI                          ahaII/bsaHI               hinPI
       mseI  bspHI                    ddeI aatII                     hhaI/cfoI
5101 AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT CGGGGAAATG TGCGCGGAAC CCCTATTTGT TTATTTTTCT AAATACATTC
     TCCAATTACA GTACTATTAT TACCAAAGAA TCTGCAGTCC ACCGTGAAAA GCCCCTTTAC ACGCGCCTTG GGATAAACA AATAAAAAGA TTTATGTAAG

                rcaI
                bspHI
            bsrBI   bsmAI                                           mboII
            aciI nlaIII                             sspI            earI/ksp632I
5201 AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT
     TTTATACATA GGCGAGTACT CTGTTATTGG GACTATTTAC GAAGTTATTA TAACTTTTTC CTTCTCATAC TCATAAGTTG TAAAGGCACA GCGGGAATAA

                                                                                          hgiAI/aspHI
                                                                                          bsp1286
                                                                               sau3AI     bsiHKAI
                                                                               mboI/ndeII[dam-)
                                                                               dpnI[dam+] bmyI
                                                                               dpnII[dam-]
          fnu4HI                                                          eco57I          apaLI/snoI
          aciI                        hphI             hphI            sfaNI mboII[dam-)  alw44I/snoI  maeIII
5301 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGCACGA GTGGGTTACA
     GGGAAAAAAC GCCGTAAAAC GGAAGGACAA AAACGAGTGG GTCTTTGCGA CCACTTTCAT TTTCTACGAC TTCTAGTCAA CCCACGTGCT CACCCAATGT
```

FIG. 10P

```
             sau3AI
             mboI/ndeII[dam-]    sau3AI
             dpnI[dam+]          mboI/ndeII[dam-]
             dpnII[dam-]         dpnI[dam+]             psp1406I      hgiAI/aspHI          aciI
            bstYI/xhoII          dpnII[dam-]            xmnI          bsp1286  tru9I       thaI
         bsrI        nspBII      alwI[dam-]             asp700        bsiHKAI  mseI        fnuDII/mvnI
     taqI  alwI[dam-]  aciI     bstYI/xhoII         mboII maeII       bmyI   ahaIII/draI   bstUI
                                                                                           bsh1236I
                                                                                           hinPI
                                                                                           hhaI/cfoI
5401 TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA GAACGTTTTC CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT
     AGCTTGACCT AGAGTTGTCG CCATTCTAGG AACTCTCAAA AGCGGGGCTT CTTGCAAAAG GTTACTACTC GTGAAAATTT CAAGACGATA CACCGCGCCA

                 scrFI
                 nciI
                 mspI
                 hpaII
                 dsaV
                 cauII
               hinlI/acyI                                              rsaI
               hgaI                  aciI                              csp6I  bsrI
               ahaII/bsaHI   bcgI   mcrI fnu4HI        ddeI            scaI hphI   maeIII      sfaNI
5501 ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACACT ATTCTCAGAA TGACTTGGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT
     TAATAGGGCA CTACTGCGGC CCGTTCTCGT TGAGCCAGCG GCGTATGTGA TAAGAGTCTT ACTGAACCAA CTCATGAGTG GTCAGTGTCT TTTCGTAGAA

                                                                                                   sau96I
                                                                                                   avaII
                                                                                        sau3AI   asuI
                                                                    haeIII/palI         mboI/ndeII[dam-]
                                                                   eaeI                 dpnI[dam+]
                                                                   cfrI                 dpnII[dam-]
                                   fnu4HI                          fnu4HI              pvuI/bspCI
      fokI  nlaIII                 bbvI        nlaIII              aciI                mcrI  mnlI
5601 ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG
     TGCCTACCGT ACTGTCATTC TCTTAATACG TCACGACGGT ATTGGTACTC ACTATTGTGA CGCCGGTTGA ATGAAGACTG TTGCTAGCCT CCTGGCTTCC

                                  nlaIII
                              sau3AI maeIII                    mspI
                              mboI/ndeII[dam-]     sau3AI    nlaIV
                              dpnI[dam+]           mboI/ndeII[dam-]
                             alwI[dam-]            dpnI[dam+]   hpaII
     aluI  aciI         nlaIII dpnII[dam-]         dpnII[dam-] bsaWI aluI                    maeIII
5701 AGCTAACCGC TTTTTTGCAC AACATGGGGG ATCATGTAAC TCGCCTTGAT CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC
     TCGATTGGCG AAAAAACGTG TTGTACCCCC TAGTACATTG AGCGGAACTA GCAACCCTTG GCCTCGACTT ACTTCGGTAT GGTTTGCTGC TCGCACTGTG
```

FIG. 10Q

```
                                                                                mspI
                                      hinPI                                     hpaII
                                    mstI                                       scrFI
                                    aviII/fspI      bsrI                  aluI nciI        tru9I      fokI
                fnu4HI          maeII hhaI/cfoI  tru9I                  rmaI   dsaV        mseI    bsrI        aciI
        sfaNI   bbvI           psp1406I          mseI                   maeI   cauII      aseI/asnI/vspI    mnlI
5801 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAACTGGCG AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG
     GTGCTACGGT CGTCGTTACC GTTGTTGCAA CGCGTTTGAT AATTGACCGC TTGATGAATG AGATCGAAGG GCCGTTGTTA ATTATCTGAC CTACCTCCGC

                                                                                                     aciI
                                      bglI                                      mspI              thaI
                                    sau96I                                      hpaII             fnuDII/mvnI
                  sau96I            haeIII/palI                               cfr10I              bstUI
                  avaII       hinPI asuI    mspI                           nlaIV  hphI        bsmAI            fnu4HI
                  asuI        hhaI/cfoI     hpaII                        gsuI/bpmI           bsaI bsh1236I     bbvI
5901 GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG
     CTATTTCAAC GTCCTGGTGA AGACGCGAGC CGGGAAGGCC GACCGACCAA ATAACGACTA TTTAGACCTC GGCCACTCGC ACCCAGAGCG CCATAGTAAC

                                                                                                           ddeI
             sau96I                                                                                  sau3AI
             asuI                                                pleI                                mboI/ndeII[dam-]
            nlaIV                                                hinfI                               dpnI[dam+]
         bsrI haeIII/palI     mnlI                       eam1105I                fokI                dpnII[dam-]
6001 CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT
     GTCGTGACCC CGGTCTACCA TTCGGGAGGG CATAGCATCA ATAGATGTGC TGCCCCTCAG TCCGTTGATA CCTACTTGCT TTATCTGTCT AGCGACTCTA

                                                                                                                 bphI
                                                                                                              rmaI
                                                                                                           sau3AI
                                                                                                           mboI/ndeII[dam-]
                                                                                                           dpnI[dam+]
          mnlI                                                                                       tru9I   dpnII[dam-]
      nlaIV                                                                    tru9I                ahaIII/draI maeI
      hgiCI         tru9I                                                      mseI            tru9I      bstYI/xhoII
      banI          mseI      maeIII                                          ahaIII/draI      mseI mseI  alwI[dam-]
6101 AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT ATATACTTTA GATTGATTTA AAACTTCATT TTTAATTTAA AAGGATCTAG
     TCCACGGAGT GACTAATTCG TAACCATTGA CAGTCTGGTT CAAATGAGTA TATATGAAAT CTAACTAAAT TTTGAAGTAA AAATTAAATT TTCCTAGATC
```

FIG. 10R

```
                                                                                                       sau3AI
      sau3AI                                                                                           mboI/ndeII(dam-)
      mboI/ndeII(dam-)                                                                                 dpnI(dam+)
      dpnI(dam+)                                                                                       dpnII(dam-)
      dpnII(dam-)                                                                                     bstYI/xhoII
      alwI(dam-)                                                                                sau3AI alwI(dam-)
      alwI(dam-)            nlaIII            maeII                                            mboI/ndeII(dam-)
     bstYI/xhoII           rcaI            tru9I                                               dpnI(dam+) mboII(dam-)
    mboII(dam-)            bspHI           mseI                   ddeI hgaI                    dpnII(dam-)
6201 GTGAAGATCC TTTTTGATAA TCTCATGACC AAAATCCCTT AACGTGAGTT TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT
     CACTTCTAGG AAAAACTATT AGAGTACTGG TTTTAGGGAA TTGCACTCAA AAGCAAGGTG ACTCGCAGTC TGGGGCATCT TTTCTAGTTT CCTAGAAGAA

                 thaI                                                                   sau3AI
    sau3AI       fnuDII/mvnI                                                            mboI/ndeII(dam-)
    mboI/ndeII(dam-)                                                                    dpnI(dam+)
    dpnI(dam+)   bstUI                                                                  dpnII(dam-)
    dpnII(dam-)  bsh1236I                                                              alwI(dam-)
    alwI(dam-)  hinPI      fnu4HI                                  aciI              mspI
   bstYI/xhoII  hhaI/cfoI  bbvI                              aciI  nspBII            hpaII      aluI
6301 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAAACCA CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT
     CTCTAGGAAA AAAAGACGCG CATTAGACGA CGAACGTTTG TTTTTTTGGT GGCGATGGTC GCCACCAAAC AAACGGCCTA GTTCTCGATG GTTGAGAAAA

          bsrI          hinPI                  rmaI          haeIII/palI
       maeIII  eco57I   hhaI/cfoI              maeI     bslI haeI                      scfI    aciI
6401 TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT
     AGGCTTCCAT TGACCGAAGT CGTCTCGCGT CTATGGTTTA TGACAGGAAG ATCACATCGG CATCAATCCG GTGGTGAAGT TCTTGAGACA TCGTGGCGGA

                                                                     scrFI
                                                                     nciI
                                  fnu4HI                             mspI                     mspI
                          alwNI   bbvI                               hpaII                    hpaII
                         bsrI   fnu4HI                               dsaV     pleI           bsaWI      hinPI
     mnlI             maeIII    bbvI     bsrI                        cauII    hinfI        maeIII      hhaI/cfoI
6501 ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG
     TGTATGGAGC GAGACGATTA GGACAATGGT CACCGACGAC GGTCACCGCT ATTCAGCACA GAATGGCCCA ACCTGAGTTC TGCTATCAAT GGCCTATTCC

                          hgiAI/aspHI
     mcrI                 bsp1286
    aciI                  bsiHKAI
   nspBII                 bmyI                                                                         hinPI
  fnu4HI                  apaLI/snoI                                                                   hhaI/cfoI
  bbvI                    alw44I/snoI  aluI                  ddeI      scfI                           haeII
6601 CGCAGCGGTC GGGCTGAACG GGGGGTTCGT GCACACAGCC CAGCTTGGAG CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAG
     GCGTCGCCAG CCCGACTTGC CCCCCAAGCA CGTGTGTCGG GTCGAACCTC GCTTGCTGGA TGTGGCTTGA CTCTATGGAT GTCGCACTCG TAACTCTTTC
```

FIG. 10S

```
                                                                                                          scrFI
                                                                                                          mvaI             scrFI
                                                                                                          ecoRII  mvaI
                                              mspI                                                        dsaV    ecoRII
                                              hpaII                                                       bstNI            dsaV
                                              bslI        fnu4HI                         hinPI   mnlI     bsaJI            bstNI
                                  aciI        bsaWI       aciI                           hhaI/cfoI    aluI apyI[dcm+]      apyI[dcm+]
6701 CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG
     GCGGTGCGAA GGGCTTCCCT CTTTCCGCCT GTCCATAGGC CATTCGCCGT CCCAGCCTTG TCCTCTCGCG TGCTCCCTCG AAGGTCCCCC TTTGCGGACC

                                                                                                                 fnu4HI
                                                                                                                 aciI
                                                                                                                 thaI
                                                                                                                 fnuDII/mvnI
                                                        taqI                                 nlaIV               bstUI
                                       mnlI   drdI    hgaI            sfaNI                aciI                  bsh1236I
6801 TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT TTTGTGATGC TCGTCAGGGG GGCGGAGCCT ATGGAAAAAC GCCAGCAACG
     ATAGAAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCGCAGCTAA AAACACTACG AGCAGTCCCC CCGCCTCGGA TACCTTTTTG CGGTCGTTGC

                          haeIII/palI
                       scrFI
                       mvaI
                       ecoRII
                       dsaV                          nlaIII
                       bstNI bslI                        nspI
         bslI          apyI[dcm+]      haeIII/palI       nspHI                      tfiI
       haeIII/palI nlaIV haeI        haeI                aflIII                     hinfI                    aciI
6901 CGGCCTTTTT ACGGTTCCTG GCCTTTTGCT GGCCTTTTGC TCACATGTTC TTTCCTGCGT TATCCCCTGA TTCTGTGGAT AACCGTATTA CCGCCTTTGA
     GCCGGAAAAA TGCCAAGGAC CGGAAAACGA CCGGAAAACG AGTGTACAAG AAAGGACGCA ATAGGGGACT AAGACACCTA TTGGCATAAT GGCGGAAACT
```

FIG. 10T

```
                                                                                                              thaI
                                                                                                              fnuDII/mvnI
                                                                                                              bstUI
                                                                                                              bsh1236I
                                                                                                             hinPI
                                                                                                             hhaI/cfoI
                                                                                                            thaI
                                                                                   hinPI                    fnuDII/mvnI
                          fnu4HI                fnu4HI                         sapI hhaI/cfoI               bstUI
                          bbvI                  bbvI  pleI                     mboII                        bsh1236I
                   bsrBI  aciI                 hinPI  hinfI                    earI/ksp632I          mnlI    bslI
        aluI       aciI  fnu4HI        mcrI    hhaI/cfoI          mnlI   aciI   haeII             aciI       aciI
7001 GTGAGCTGAT ACCGCTCGCC GCAGCCGAAC GACCGAGCGC AGCGAGTCAG TGAGCGAGGA AGCGGAAGAG CGCCCAATAC GCAAACCGCC TCTCCCCGCG
     CACTCGACTA TGGCGAGCGG CGTCGGCTTG CTGGCTCGCG TCGCTCAGTC ACTCGCTCCT TCGCCTTCTC GCGGGTTATG CGTTTGGCGG AGAGGGGCGC

                 tru9I   aluI
       haeIII/palI       pvuII                                                      tru9I                   nlaIV
      eaeI tfiI   mseI   nspBII                                       hinPI         mseI      maeIII        hgiCI
      cfrI hinfI aseI/asnI/vspI               bsrI     aciI           hhaI/cfoI   aseI/asnI/vspI mnlI       banI
7101 CGTTGGCCGA TTCATTAATC CAGCTGGCAC GACAGGTTTC CCGACTGGAA AGCGGGCAGT GAGCGCAACG CAATTAATGT GAGTTACCTC ACTCATTAGG
     GCAACCGGCT AAGTAATTAG GTCGACCGTG CTGTCCAAAG GGCTGACCTT TCGCCCGTCA CTCGCGTTGC GTTAATTACA CTCAATGGAG TGAGTAATCC

       scrFI
       mvaI
       ecoRII
       dsaV
       bstNI
       apyI(dcm+)             mspI                      aciI                                                xmnI
      bsaJI                   hpaII                    bsrBI                             aluI     nlaIII    asp700
7201 CACCCCAGGC TTTACACTTT ATGCTTCCGG CTCGTATGTT GTGTGGAATT GTGAGCGGAT AACAATTTCA CACAGGAAAC AGCTATGACC ATGATTACGA
     GTGGGGTCCG AAATGTGAAA TACGAAGGCC GAGCATACAA CACACCTTAA CACTCGCCTA TTGTTAAAGT GTGTCCTTTG TCGATACTGG TACTAATGCT

      tru9I
      mseI
     aseI/asnI/vspI
7301 ATTAA
     TAATT

>length: 7305
```

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/09576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/64 C12N15/67 C12N15/85 C12N9/72 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DNA CLONING, VOLUME III, EDITED BY D.M. GLOVER, 1987 IRL PRESS, OXFORD, GB;, pages 189-212, A.M.C. BROWN AND M.R.D. SCOTT 'Retroviral vectors'	1-3,7,8
Y	see page 192, line 7 - page 196, line 5; figures 2,3	5,6, 9-12, 16-21
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1995	08.12.95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Hornig, H

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Y	pZIP-Neo SV(B)1 see figure 1	5,6, 9-12, 16-21
Y	MOL. CELL. BIOL., vol. 5, no. 3, March 1985 ASM WASHINGTON, DC,US, pages 431-437, A.D. MILLER ET AL. 'Generation of helper-free amphotrophic retroviruses that tranduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene' see page 432, right column, line 5 - page 436, right column, line 7; figure 1	5,6, 9-12, 16-21
Y	WO,A,94 05784 (US) 17 March 1994 see the whole document	5,6, 9-12, 16-21
Y	EP,A,0 215 548 (ZYMOGENETICS INC ;UNIV WASHINGTON (US)) 25 March 1987 see the whole document	5,6, 9-12, 16-21
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROC. NATL.ACAD SCI., vol. 86, February 1989 NATL. ACAD SCI.,WASHINGTON,DC,US;, pages 1041-1045, M. VIVAUD ET AL. 'A 5' splice-region G-C mutation in exon 1 of the human beta-globin gene inhibits pre-mRNA splicing: A mechanism for beta+-thalassemia' see the whole document -----	1-4

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		NO-B-	174934	25-04-94
		SG-A-	3994	10-06-94
		US-A-	4965199	23-10-90